# COMPARATIVE EFFECT OF EDTA, EGTA, CITRIC ACID AND MTAD SOLUTIONS ON SMEAR LAYER REMOVAL AND MICROHARDNESS ON INSTRUMENTED ROOT CANAL DENTIN – AN INVITRO STUDY

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## CERTIFICATE

*This is to certify that this dissertation titled* ***"Comparative effect of EDTA, EGTA, Citric acid and MTAD solutions on smear layer removal and microhardness on instrumented root canal dentin- An invitro study"****,is a bonafide record of the work done by Dr.Manu Unnikrishnan under our guidance during his/her post graduate study during the period of 2010-2013 under* **THE TAMIL NADU Dr.MGR MEDICAL UNIVERSITY, CHENNAI,** *in partial fulfillment for the degree of* **MASTER OF DENTAL SURGERY IN CONSERVATIVE DENTISTRY & ENDODONTICS, BRANCH IV.**

*It has not been submitted (partial or full) for the award of any other degree or diploma.*

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# ABSTRACT

# ABSTRACT

**Introduction** – The cleaning and shaping of root canal system has undergone paradigm shift from one fulfilling a prime debridement function to one more on gaining radicular access for action of irrigant and three dimensional obturation of prepared root canal. Chemomechanical preparation of root canal system helps in removing organic as well as inorganic debris and microorganisms from the infected root canals. One desirable property of root canal irrigants is to remove smear layer from instrumented root canals with no adverse effects on remaining tooth structure.

**Aims and Objectives-** This study was done to evaluate and compare the smear layer removal property and dentin microhardness by the use of 17 % EDTA, 17% EGTA, 10 % citric acid, MTAD, and alternating use of 5.25% sodium hypochlorite and 17% EDTA as a final rinse for 5 minutes on extracted mandibular single rooted premolars after rotary instrumentation.

**Methodology-** Sixty extracted non carious single rooted mandibular premolars divided into 5 groups of 12 each were instrumented using rotary instrumentation to apical enlargement of ISO 30 /0.06 size. After initial rinse during instrumentation using 5.25 % sodium hypochlorite, the final rinse was performed with 10 ml of 17% EDTA, 17 % EGTA, 10 % citric acid, MTAD, 5.25% Sodium hypochlorite and 17% EDTA. The samples were evaluated for smear layer removal using scanning electron microscopy and dentin microhardness using Vickers microhardness testing machine.

**Results and Observations–** The sequential use of 5.25% sodium hypochlorite and 17% EDTA as final rinse was found to be a more effective irrigation regimen for smear layer removal and this protocol when followed resulted in lesser reduction in dentin microhardness. 17 % EDTA when applied for 5 minutes was more effective in smear layer removal from cervical and middle portions of root canal but created dentinal erosion more in cervical and middle third of root canal. . 17 % EGTA caused least reduction in dentin microhardness but was less effective in removing smear layer particularly the smear plugs. 10 % citric acid was effective in removing smear layer from cervical, middle and apical portions. Application of higher volumes and time was required to effectively remove smear layer and moreover citric acid caused substantial reduction in dentin microhardness. MTAD was effective in removing smear layer effectively from cervical and middle portions but least effective in apical third of instrumented root canals.

**Conclusion –** The alternating regimen of irrigation with 10ml of 5.25 % sodium hypochlorite and 17% EDTA for 5 minutes was found to be more effective in smear layer removal and produced less dentinal erosion and thus lesser reduction in dentin microhardness when compared with the other irrigants.

**Clinical Significance** – Cleaning and shaping of root canals supplemented by irrigation provides a dentin surface that is free of smear layer and debris. Along with smear layer removal ability, the irrigant should have least demineralizing action on dentin. An irrigant meeting these prerequisites provides for the success of root canal treatment

# INTRODUCTION

The phase of preparing and debriding the root canal is undoubtedly the most important, the most complex, and the most delicate part of endodontic treatment. It is difficult to imagine how one can completely obturate a canal that has not been adequately cleaned and disinfected. Over the years, canal preparation has been described by a variety of names including “enlargement”, mechanical preparation” and “instrumentation”.In modern Endodontics the emphasis is on biological and anatomical problem, hence cleaning and shaping are more correct terms. Schilder introduced these terms to endodontics in 1974. [1]

The shaping of root canal system has undergone a paradigm shift from one fulfilling a prime debriding function to one more regarded as gaining a radicular access to complex root canal systems, for the irrigant and root filling material. It has been found by using high resolution computed tomography that nearly 35 -53 % of the root canal surface remained uninstrumented, showing that instrumentation alone is inadequate. [2]

Thus when “preparing” a root canal system , it is infact cleaned of all inorganic debris, organic substances, and microorganisms and it is shaped to facilitate placement of a permanent three dimensional filling. When files produce shaping, it is essential that irrigants clean a root canal system. [1]

Those teeth having infected root canals have bacteria concentrated in the coronal and apical portion of the root canal system. The Transmission electron microscopy observation of carious teeth reveals that most of the flora in the apical 5 mm of the root canal is suspended in apparently moist canal lumen. [2]

The instrumentation, depending upon the design of instruments used, remove some of the residual tissue by engaging it, some will be compacted and burnished against the root canal walls. Whenever dentine is being cut using hand or rotary instruments, the mineralized tissues does not get shredded or cleaved but are shattered to produce considerable quantities of debris. The nature of smear layer created with current nickel – titanium rotary techniques may considerably vary from that formed using stainless steel instrumentation because of the different mechanical and chemical forces that comes into play. [2]

The identification of the smear layer using scanning electron microscope SEM was first reported by Eick. Scanning electron microscope studies of cavity preparations by Brannstrom and Johnson demonstrated a thin layer of grinding debris. They estimated it to be 2-5 µm thick. The first researchers to report the smear layer on the surface of instrumented root canals were McComb and Smith in [3]

It has been observed that bacteria could remain in the smear layer and in the dentinal tubules despite instrumentation of the root canal and thus they may survive and multiply and can grow into dentinal tubules. The chemomechanical cleansing is often supported by the use of disinfectants. Few others believe in the fact that the presence of the smear layer may block the antimicrobial effects of intracanal disinfectants into the tubules. [4]

Assuming that smear layer removal is a desirable property of root canal irrigants, an ideal root canal irrigant should be biologically compatible, chemically able to remove both organic and inorganic substrates, be antibacterial, demonstrate good surface wetting, have no adverse effects on remaining tooth structure, and be easy to use and effective within clinical parameters.[2]

The current methods of smear layer removal includes chemical, ultrasonic and laser techniques. The quantity of smear layer removed by a material is related to its pH and time of exposure. A number of chemicals have been investigated as irrigants to remove smear layer. According to Castelluci a working solution is the one which is used to clean the canal, and an irrigation solution is one which is essential to remove debris and smear layer created by instrumentation process.[3]

Removal of the smear layer is accomplished with acids or other chelating agents such as ethylenediamine tetracetic acid (EDTA) following cleaning and shaping. Irrigation with 17% EDTA for one minute followed by a final rinse with sodium hypochlorite is a recommended method.[5]

Chelators remove the inorganic components leaving the organic tissue elements intact. Sodium hypochlorite is then necessary for removal of the remaining organic components. Citric acid has also been shown to be an effective method for removing the smear layer as has tetracycline. [6]

An alternative method for removing the smear layer employs the use of a mixture of a tetracycline isomer, an acid, and a detergent (MTAD) as a final rinse to remove the smear layer. The effectiveness of MTAD to completely remove the smear layer is enhanced when low concentrations of NaOCl are used as an intracanal irrigant before the use of MTAD. A 1.3% concentration is recommended. [7]

Ethylene glycol-bis (β-aminoethyl ether) – N,N,N,N – tetra acetic acid (EGTA) has been used by researchers at low concentration when a medium free of calcium is needed. A lot of research in medicine on chelating action to heavy metals that contaminate patients had been done. EGTA has been used for this purpose. Schmid & Reilley reported that EGTA can bind calcium more specifically. [8]

In this study 5.25 % sodium hypochlorite is used as the common irrigant during instrumentation in all the groups. Alternating use of 17 % EDTA and 5.25 % sodium hypochlorite is used as positive control. Distilled water is used as negative control. In Group I, 10 ml of 17% EDTA was used as the final rinse, in Group II, 10 ml of EGTA was used as the final rinse, in Group III, 10 ml of MTAD was used as the final rinse and in Group IV, 10 ml of 10% citric acid was used as the final rinse. The irrigating solutions when in contact on dentine cause alterations on dentine and enamel. The studies on modes of action and efficiency of various chemical irrigating solutions have shown to have effect on both organic and inorganic components of root canal dentin.

Dentin microhardness depends on the amount of calcified matrix per $mm^2$ and its determination provides indirect evidence of mineral loss or gain in the dental hard tissues. Surface changes evaluation of dental hard tissues for alteration in calcium – phosphorous ratio has been done by methods like microhardness measurement, micro radiographic assessments, scanning electron microscopic methods, energy dispersive spectrometric analysis and surface roughness testing. The microhardness measurement was one of the simplest non-destructive mechanical characterization methods. The suitability and practicality of Vickers hardness test for evaluating surface changes is adopted in this study.[9]

The efficacy of smear layer removal in cervical, middle and apical portion of instrumented root canals of extracted mandibular premolars by 17 % EDTA, 17% EGTA, MTAD, 10% citric acid when used as a final rinse and effect of the solutions on dentine microhardness is evaluated.

# AIMS AND OBJECTIVES

**Aims** -This study was done to examine the smear layer removal property and dentin microhardness by the use of 17 % EDTA, 17% EGTA , 10% citric acid, MTAD and alternating use of 5.25 % sodium hypochlorite and 17 % EDTA as a final rinse for 5 minutes on extracted human mandibular single rooted pre molars after rotary instrumentation.

**Objectives -** The effect of these solutions on smear layer removal and the dentin microhardness were compared.

# REVIEW OF LITERATURE

**Jay.K.Jaylor et al. (1997) [10]** examined the effect of obturation technique, sealer and presence of smear layer on coronal microleakage. The comparison between smear layer removal and smear layer not removed was done. Results showed less leakage when smear layer was removed. AH 26 displayed less leakage than Roth's 811 sealer. Vertical compaction reduced leakage when compared to lateral condensation, ultrafil and thermafil obturations. The study shows that removal of the smear layer resulted in decreased coronal leakage regardless of obturation technique used. Wennberg and Ostavik reported that when compared to zinc oxide eugenol sealers, the resin sealers had increased adhesion to root canal dentine. The Ketac – Endo and AH-26 showed less dye leakage when smear layer was removed. Thermafil technique without vertical condensation was susceptible to coronal leakage. This study reported that leakage significantly decreased with vertical condensation around the plastic carrier.

**Siriporn Timpawat et al.( 1998) [11]** studied the effect of two root canal sealers (Ketac-Endo Glass Ionomer sealer and Zinc oxide eugenol cement) on apical seal in roots obturated with thermafil obturators. The study showed the root canal sealers improved the apical seal of thermafil regardless of the presence or absence of a smear layer. There was no clear difference in the apical leakage between using a zinc oxide eugenol sealer and Ketac –Endo with or without the smear layer. As the studies relating removal of smear layer showed better adaptation of gutta percha were focused on only the coronal and middle third of the canal . Thus the effect of adaptation might be poor in the apical third where some smear layer was left intact.

**F.H.Takeda et al.(1999)[12]** stated that 17% EDTA when used as final flush was not effective in smear layer removal whereas acidic solutions like 6% phosphoric acid and 6% citric acid produced demineralization of dentin. $CO_2$ and Er.YAG laser were more efficient in smear layer removal than EDTA or acidic solutions. The phosphoric acid and citric acid removed smear layer from the root canal , but it decalcified and caused softening of dentine to a depth of 10 to 15µm. The low pH (1.5) could cause adverse effects on the periapical tissues. The dentine demineralization caused by 17% EDTA was less compared acidic solutions. The smear layer removal produced with citric acid were similar when compared to EDTA. Nd:YAG laser irradiation created very clean root canals with smear layer removed or melted and recrystallized. The root canals showed charred, melted, recrystallized, or glazed smear layer. The Er:YAG laser showed clean surfaces, free of a smear layer having open dentinal tubules without any melting.

**Economides et al.(1999)[13]** evaluated the influence of the smear layer on the sealing ability of two root canal sealers - Roth 811 and AH 26 over a period of 16 weeks. Roth 811 is a zinc oxide eugenol based sealer and AH 26 is a resin based sealer. The results indicated that removal of smear layer improved quality of sealing when AH 26 was used and with the use of Roth 811, no statistically significant differences were detected. Smear layer removal improves adaptation and binding of sealers on root canal walls especially in case of AH 26 .In this study final flush with 1% sodium hypochlorite was not capable of smear layer removal and the combination of 1% sodium hypochlorite and EDTA (3ml /3min) was able in smear layer removal. The smear layer removal enhanced the penetration of sealers into dentinal tubules.

**M.F. Bertrand et al.(1999)[14]** found that the middle and apical thirds of canals were cleaner using the complete Quantac sequence when compared using K.files. The Quantac system is designed to collect debris and smear layer and carry it out of canal system. The conventional manual instrumentation with K files and handpiece driven Quantac system both were effective in removing smear layer from coronal third of the canals.

**V.Kytridou et al. (1999)[15]** using the thermafil obturation technique showed extrusion of material beyond apical foramen in 84% of the specimens where as only 43% of specimens obturated with system B technique showed extrusion. In this study, the removal of smear layer was not a factor influencing the adaptation of obturation and was not a factor contributing to apical leakage. Both the Themafil and System B techniques showed acceptable toot canal fills in the coronal,middle and apical third.

**Miriam.F Zaccaro Scelza et al.(2000)[16]** used three different solutions as final irrigation to assess the degree of smear layer removal. The final 4 minute irrigation included three groups

Group I – 10ml 1% of NaOCl + 10ml of 10% citric acid + 10ml of distilled water.

Group II – 15ml of 0.5% of NaOCl + 15ml of EDTA-T , Tergentol (sodium lauryl ether sulfate)

Group III – 10ml of 5% of NaOCl + 10ml of 3% $H_2O_2$ + 10ml of 5% NaOCl

Scanning electron microscopic photomicrographs evaluation for mean number of visible open dentinal tubules showed largest number of visible tubules in cervical third followed by middle and apical thirds. The volume of solution as well as chemical properties of irrigating solutions were important determining factors which aids in removal of debris. Adding Tergentol (sodium lauryl ether sulfate ) to EDTA caused significant reduction in surface tension, resulting in deeper penetration of solution for removal of smear layer.

**R.Di Lenarda et al. (2000) [17]** evaluated the cleansing and smear layer removal ability of alternate canal irrigation with citric acid and sodium hypochlorite. A substitution of EDTA with aqueous solution of citric acid solution was proposed by Yamaguchi et al. Yamaguchi evaluated antibacterial capability of citric acid at all tested concentrations. Calcium extraction from a resin mixture was more effective using citric acid solutions. Irrigation of canal using citric acid and sodium hypochlorite causes neutralization of the previous irrigant as stated by Di Lenarda et al. In this study three groups were subdivided on the basis of the method of irrigation. (i) sodium hypochlorite was used alone after every instrumentation step. (ii) alternated with 19% citric acid solution (iii) a combination of 15% EDTA and Cetrimide solution. Results obtained suggest that at the apical third of treated canals citric acid was significantly better when compared with EDTA. Within the manually instrumented group involving more number of instrument steps and a longer exposure to irrigating solutions the EDTA group was superior to citric acid. In the group treated mechanically with ProFile .04 taper instruments, more effective was citric acid.

**Von Fraunhofer et al.(2000) [18]** evaluated effects of canal instrumentation and effects of smear layer on leakage in filled teeths. Results indicated less microleakage when smear layer was removed. The canals obturated using thermoplasticized gutta percha showed less leakage than lateral condensation. Rotary driven instrumented canals had less leakage than hand instrumented canals. Nickel-titanium instruments has superiority with regard to elasticity and resistance to torsional fracture when compared to stainless steel endodontic files. The conservation of original path of curved root canals and accuracy of final apical diameter produced by rotary – instrumentation resulted in less leakage.

**Michael S.O'Connell et al. (2000)[19]** evaluated three solutions of EDTA- 15% concentration of the alkaline salt, a 15% concentration of the acidic salt, and a 25% concentration of the alkaline salt were used for smear layer removal. The results showed none of EDTA solutions by themselves were effective in complete removal of smear layer. It was found that when used in combination with NaOCl, all solutions of disodium and tetra sodium EDTA were equally effective at removing smear layer in coronal and middle thirds but not as effective in the apical third of root. The alkaline tetra sodium salt was more cost effective as the commonly used disodium salt of EDTA. The pH is a major factor determining the ratio of ionized to nonionized forms of EDTA in solution. The solutions when used at higher concentrations lead to increased demineralization properties. When root canals were irrigated with alternatively by sodium hypochlorite and EDTA , the dentinal tubules were open in cervical and middle thirds, and amorphous smear layer was evident in the apical third.

**J.T.Marais et al.(2000)[20]** evaluated a new product named electro-chemically activated water and compared it to NaOCl for its cleaning effect on root canal walls. The results showed that electro chemically activated water produced markedly clean surfaces and removed smear layer in large areas. The cleaning efficacy of electro chemically activated water was superior to NaOCl. The physical and chemical nature of electro-chemically activated water is not known. Russian scientists developed a process through which electro-chemically activated water (ECA) was produced with new anode – cathode system. Elecro-chemically activated water is produced from tap water, salt and electricity. This solution cleaned root canal wall in remarkable way, removing the smear layer. Exposure of collagen fibres and fibrils indicate that dentine was decalcified. Anolyte used was of a neutral pH, the catholyte of pH 9.8.

**S.D.Gilbert et al.(2001)[21]** studied by comparing coronal bacterial and ink leakage following obturation with three different techniques after smear layer removal. Gutta-percha can be delivered to the canal by four basic techniques . (i) cold compaction of gutta-percha (ii) the compaction of gutta-percha been heat softened in the canal and then cold compacted (iii) thermoplasticized and injected into the system and then cold compacted (iv) compaction of gutta-percha that has been placed and softened by mechanical means.The results indicated that when challenged with bacteria, the vertical compaction technique exhibited less leakage than lateral compaction. The thermafil obturation had numerically less leakage than lateral compaction. An interesting observation was the fact that all teeth that exhibited bacterial leakage also leaked when challenged with India ink. When bacterial leakage values were compared , teeth obturated by lateral compaction and Thermafil were

11.56 and 5.39 times more likely to leak than vertical compaction. Teeth obturated by lateral compaction was 2.14 times more likely to leak than Thermafil.

**Hata et al.(2001)[22]** evaluated effectiveness of oxidative potential water (O.P.W) on its ability to remove smear layer and debris from instrumented root canals. Oxidative potential water (O.P.W) was used extensively in Japan for household and agricultural disinfection because of the safety and bactericidal effectiveness. Scientific basis for development of the Oxidative potential water was that microorganisms cannot survive in aqueous environment with low pH (less than 3) and high oxidation – reduction potential. The study indicated that ultrasonic irrigation with oxidative potential water was less effective in removing smear layer than syringe irrigation with oxidative potential water. The study demonstrated the ability of oxidative potential water to remove the smear layer after root canal instrumentation and found that irrigation with Oxidative potential water and a syringe was deemed useful for root canal irrigation. Study showed that ultrasonic irrigation with Oxidative potential water was less effective in smear layer removal than syringe irrigation with oxidative potential water.

**D.Grigoratos et al. (2001)[23**] studied the effect on mechanical properties of dentine by sodium hypo chlorite and calcium hydroxide. Sequential exposure to sodium hypochlorite (3% or 5%) and saturated calcium hydroxide solutions on flexural strength and modulus of elasticity of standardized dentine bars were investigated. Treatment of dentine bars with 3% and 5% sodium hypochlorite solutions showed significant decrease in modulus of elasticity and flexural strength The modulus of elasticity as well as flexural strength of 5% sodium hypochlorite treated specimens were higher than those treated with 3% sodium hypochlorite. The results were that dentine bars exposed to $Ca(OH)_2$ had no effect on modulous of elasticity but reduction in flexural strength whereas dentine bars treated with 3% and 5% NaOCl solutions had significant decrease in modulous of elasticity and flexural strength.

**J.T.Marais et al.(2001)[24]** stated that there are two types of electro chemically activated water used as irrigants. The Anolyte and Catholyte. The Anolyte has high oxidative potential. Catholyte is an alkaline solution with high reduction potential. The antimicrobial activity of anolyte has been reported. The study evaluated the effect of electro chemically activated water on selected group of anaerobic bacteria. The result showed that electrochemically activated water failed to destroy all of the bacteria within the root canals. Explanation for failure of Electro-chemically activated water to destroy bacteria is that in this study only anolyte was used , as opposed to the use of catholyte followed by anolyte. Catholyte is claimed to have cleaning or detergent effect. Another reason may be at the time of preparation of the Electro- chemically activated solutions , a technical failure of the unit may have caused an altered Electro-chemically activated solution.

**Sim et al.(2001)[25]** evaluated the mechanical properties of dentine and tooth surface strain after sodium hypochlorite irrigation. Although sodium hypochlorite remains the irrigant of choice, the study using 5.25% concentration showed negative effect on the properties of teeth. A decrease in flexural strength, an increase in surface strain-tensile strain by 15.9% and compressive strain by 33.5% was found. The study indicated the reduction in flexural strength and elastic modulous of dentine. Change in physical properties was explained by the loss of the organic matrix within the dentine. During two 30 minute period irrigation with 5.25% sodium hypochlorite, the organic portion of the dentine was removed to an extent that further removal of organic matrix affect the residual strength of the tooth. An aggressive irrigation regimen was selected in this study in order that the effect was guaranteed. Study demonstrated that 5.25% sodium hypochlorite reduces flexural strength and elastic modulus of dentin.

**G.E.Evans et al.(2001)[26]** evaluated the effectiveness of preparation technique and sodium hypochlorite in removal of pulp and predentine from root canals of posterior teeth. The importance of preparation technique had a more dominant influence than the irrigant. The isthmus areas were untouched by both preparation techniques (Step back preparation and Ni Ti rotary preparation technique). The residual pulp tissue was more at apical portion for step back preparation than for rotary preparation. A higher number of no debris score was found in the Quantac preparations . Inference is both the preparation technique and irrigant play an important role in debridement . Frequency of remaining pulp tissue scores for all groups were at 1 mm level. Overall finding was one of pulp tissue being compacted against apical stop and some extruded. If patency filing procedure were used , the pattern might been different.

**Antonio M. Cruz-Filho et al (2001) [8]** studied the effect of EDTAC (ethylene diamine tetra acetic acid plus cetavlon), CDTA (cyclohexane-1,2 diamine tetra acetic acid), EGTA (ethylene glycol-bis-β amino ethylether N,N,N’,N’-tetra acetic acid) on microhardness of radicular dentine of cervical third of human teeth was assessed. The means obtained for each of the 5 samples in different areas of dentin were 42.26 to 45.84 for water, 36.10 to 41.02 for 15% EDTAC , 35.22 to 41.72 for 1% CDTA , and 33.54 to 38.62 for 1% EGTA. Test showed statistically significant difference for the control solution compared with EDTAC,CDTA, and EGTA, which were similar. Yamamoto et al. stated that EDTA chelates calcium and magnesium ions and that EGTA chelates calcium ions only.The results showed that the three chelating agents significantly reduced microhardness when compared with water. There was no statistically significant difference among the three solutions.

**Semra Calt et al. (2002) [27]** evaluated the effectiveness of EDTA in smear layer removal and on structure of dentine after 1 and 10 min application of 10ml of 17 % EDTA solution. Middle third of single root teeth cut longitudinally into two equal segments, were irrigated for 1 and 10 minutes. The results showed that 1 min EDTA irrigation was effective in removal of smear layer. 10 min application of EDTA caused excessive peritubular and intertubular dental erosion. Disodium salt of EDTA is the most effective chelating agent bwith prominent lubricating properties and used widely in endodontics. Used to enlarge canals, remove the smear layer, and prepare the dentinal walls for better adhesion of filling materials. EDTA solution has strong demineralization effect, cause enlargement of dentinal tubules , softening of dentin, and denaturation of collagen fibres. Prolonged application of EDTA where specimens were irrigated with 17% EDTA for 10 minutes , followed by 5% sodium hypochlorite showed smear layer completely removed , however erosion of the intertubular dentin and peritubular dentin was seen. Excessive erosion led to conjugation of tubular apertures and widening of tubular diameters.

**Ahmet Serper et al.( 2002)[28]** studied the effects of concentration and pH of EDTA on the dentinal demineralization was studied, Demineralizing effects of EDTA solutions at 10% and 17% concentrations at pH 7.5 and 9.0 was determined by measuring the liberated phosphorous at 1,3,5,10 and 15 min after exposure. The phosphorous liberated from dentine was greater with increased concentration of EDTA and increased time of exposure. Cury et al. reported that the effect of EDTA solutions on the demineralization of dentin is influenced by pH , the greatest demineralizing effect of EDTA solution was achieved at pH between 5.0 and 6.0. EDTA was found to be effective at a neutral pH than when it is at pH 9.0.

**Balagi.T.S et al.(2002)[29]** evaluated when 17% EDTA and 4% NaOCl were used as alternative irrigants and 17% EDTA and 5% ethylene diamine were used as a single mixture. Both were effective in complete removal of smear layer and debris at coronal, middle and apical thirds of root canal. When 17% EDTA or 4% NaOCl was used alone it could remove inorganic and organic components of smear layer only. Specimens that were irrigated with alternate use of 17% EDTA and 4% sodium hypochlorite showed more removal of smear layer. Specimens treated with single mixture of 17% EDTA and 5% Ethylenediamine showed maximum removal of smear layer and debris Sodium hypochlorite is a reducing agent and has ability to remove loose superficial debris and dissolve organic debris by release of hypochlorous acid that reacts with insoluble protein to form soluble peptides.

**B.E.Mayer et al. (2002)[30]** studied the effectiveness of rotary instruments and ultrasonic irrigation on debris and smear layer. Two tips of different design and material, a cutting stainless steel K files and blunt Ni Ti wire were compared as transmitters of ultrasonic energy to activate irrigants. The activation of irrigants did not significantly reduce smear layer and debris, but anti bacterial effects of ultrasonically activated irrigants was more effective. The larger apical shapes improves debridement and disinfection of canals as stated by Abou-Rass and Paccinino. Two tips of different instrument design and material , a cutting stainless steel K file and blunt Ni-Ti wire were compared.

**M.Hulsmann et al.(2002)[31],** M.Heckendorff and F.Schafers studied the effects of three chelating agents namely - Calcinase slide, Glyde file and R.C.prep on root dentine was evaluated. No differences in changes in microhardness were found regardless of time of application (2.5, 5 and 10 min) with regard to canal cleanliness for all pastes. The cleanliness decreased from coronal to apical third and the specimens treated with calcinase slide showed higher weight loss than R.C.prep after 6 and 9 min working time. Glyde file was significantly superior to R.C prep after 6 min. To enhance the degree of cleanliness copious irrigation of canal using irrigants has been suggested including chelating agents as stated by Harrison et al . The chemical and pharmacological properties of EDTA preparations were evaluated. The EDTA containing agents should be used between 1 and 5 min. Apical extrusion of chelators should be avoided.

**J.Derek White et al.(2002)[32]** evaluated the effect of commonly used endodontic materials calcium hydroxide, sodium hypochlorite, and mineral trioxide aggregate on root dentine. The force required to fracture respective sample were applied. The load was applied by Instron at the rate of 0.02mm/min. The results observed a weakening of the samples. The weakening observed was caused by break down of protein structure caused by the alkalinity of the materials used. MTA samples showed greatest range in force required to fracture.

.**Mahmoud Torabinejad et al.(2003)[33**] studied the effect of various concentrations of sodium hypochlorite as an intracanal irrigant before use of MTAD as a final rinse to remove smear layer. The canals were treated for 2 minutes with 5ml of irrigating solutions as final rinse. The solutions used were 5.25% NaOCl, sterile distilled water, 17% EDTA, MTAD. The results showed the use of MTAD as irrigant left some odontoblastic process in the dentinal tubules and some organic debris. As high concentrations of NaOCl are more toxic, the difference in ability to dissolve necrotic tissue was not significant between 1.3%, 2.6% and 5.25% NaOCl. It seems precedent to use lowest concentration of NaOCl (1.3%) followed by MTAD as final rinse to remove efficiently the smear layer. MTAD is an acidic solution with a pH of 2.15. Yamada et al. found a final flush with EDTA followed by sodium hypochlorite was effective method to remove smear layer. A study using application of various concentrations of sodium hypochlorite and final rinse with MTAD, a chemical reaction occurs between sodium hypochlorite and residual MTAD, resulted in brown solution in root canals caused by release of doxycycline present in MTAD solution.

**Mohmoud Torabinejad et al.(2003)[34]** studied the use of a mixture of a tetracycline isomer, an acid and a detergent (MTAD) as a final rinse on the surface of instrumented root canals. The results of presence or absence of smear layer and amount of erosion at coronal, middle and apical portion of canals indicated that MTAD is an effective solution for removal of smear layer and did not result in change in structure of dentinal tubules when used as a final rinse. Tetracycline is a broad spectrum antibiotic that is effective against wide range of microorganisms. Tetracycline is bacteriostatic in nature , because in the absence of bacterial cell lysis, antigenic by-products are not released, It has low pH and act as a calcium chelator and cause enamel and root surface demineralization. In addition it has substantive property. Tetracycline shown to enhance healing after periodontal therapy.

**Deborah Clark-Holke et al. (2003) [35]** evaluated if the smear layer affects the passage of bacteria through obturating material. Smear layer forms an intermediate barrier that interferes the adhesion and penetration of sealers into dentinal tubules. Cergneux et al. found less dye leakage when smear layer was removed with either EDTA or ultrasound. In the in vitro study where lateral condensation and AH 26 sealer were applied, removal of smear layer resulted in no bacterial leakage.

**A.Khademi et al.(2004)[36]** stated that both EDTA 17% and citric acid 7% removed the smear layer but 17% EDTA was more effective and the superiority was observed in middle and apical regions. In the cervical region both solutions showed no significant differences. The middle third region had highest degree of cleanliness compared to other areas and apical third had the least. Results of this study states that using both EDTA and citric acid in smear layer removal provide satisfactory results.

**O.Yoldas et al.(2004) [37]** studied the effect on microhardness of root canal dentin after contact with $Ca(OH)_2$ glycerine combination and $Ca(OH)_2$ distilled water combination at different time intervals. No reduction in dentine microhardness occurred after 24h. Reduction in microhardness for both combinations were observed after 3 and 7 days. Comparison of both combinations indicated that reduction in microhardness after $Ca(OH)_2$ glycerine treatment was significantly greater than after $Ca(OH)_2$ distilled water combination. The greater reduction in dentin microhardness found after calcium hydroxide-glycerine combination was explained by different penetration ability of the two combinations into dentinal tubules.

**Brent.J.Crumpton et al.(2005)[38]** stated that 1ml of EDTA with a contact time of 1 minute was effective in removal of smear layer as 10ml. The use of EDTA over 1ml resulted in no further debris removal. The study result showed that 1ml of EDTA was as effective in removing the smear layer as 10ml. It may be hypothesized that the effects of EDTA are a function of contact time with no relation to volume of irrigation.

**Hale Ari et al. (2005)[39]** evaluated the mineral contents of root canal dentin after irrigating with 0.2% chlorhexidine gluconate for 15min, 3% $H_2O_2$ for 15min, 17% EDTA for 15min, 5.25% NaOCl for 15min, 2.5% NaOCl for 15min and distilled water as control. The levels of five elements (calcium, phosphorus, magnesium, potassium, sulphur) were analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-AES) technique. Result showed that a significant decrease in Ca level with all irrigation solutions except 5.25% sodium hypochlorite. This study found that sodium hypochlorite, hydrogen peroxide, or combination of sodium hypochlorite and hydrogen peroxide have negative effects on bond strength of adhesive cement to root canal dentin.

**Kishor Gulabivala et al.(2005)[2]** evaluated the effects of mechanical and chemical procedures on root canal surfaces due to altered chemical composition of dentin as a result of application of irrigants and dressing. The NaOCl causes depletion of organic element of dentin (collagenous component) and mineral component is left relatively intact. When irrigation is alternated with EDTA, the degradation of hydroxyapatite occurs and results in change in visco elastic properties. The aggressive irrigation with NaOCl and EDTA cause surface erosions in dentine.

**C.S.Teixeira et al.(2005)[40]** studied the combined use of EDTA and NaOCl and found to be effective in removing smear layer from cervical and middle third for all times of application for 1,3 and 5 minutes. In the apical third the effective removal of smear layer was decreased when irrigated for 1 minute. The entrapment of air bubbles prevent the total filling of irrigant in the apical third. The mechanical stirring with lentulospiral removes air bubbles and favors improved contact of EDTA to canal walls. Irrigating canals for 5 minutes, Lopes et al. reported that the mechanical stirring of EDTA for 2 minutes using lentilo spiral allowed for near removal of smear layer from apical third. On account of reduced dimension of root canal, air bubbles remain trapped and prevent total filling with the irrigant. Mechanical stirring with lentilo spiral removes the air bubbles and favours improved contact of EDTA with the canal walls.

**Franklin R. Tay et al.(2006)[41]** evaluated the structure of mechanically instrumented intra radicular dentine after irrigation with NaOCl as initial rinse and Bio Pure MTAD as final rinse were examined using transmission electron microscopy. Sterile water and EDTA as final rinse were used as respective positive and negative controls. The results showed 2 to 5 μm thick smear layer produced mechanically were removed by EDTA and Bio Pure MTAD under agitation. Both irrigants created a zone of demineralized collagen matrices in eroded dentin and around dentinal tubules. The thickness of these demineralized zones were comparable to those formed on crown dentin after phosphoric acid etching for 15 seconds. The acidity of Biopure MTAD is similar to mild self-etching primers used in dentin bonding. A 5 minute irrigation is long compared with 30 -60 seconds etching time recommended for self-etching primers. Mechanical agitation for enhanced efficacy of smear layer removal and replenishing of fresh irrigants during subsequent rinsing may have also contributed to formation of thick demineralized dentin zones. Because demineralized collagen matrices are susceptible to collapse after air drying, their presence after endodontic irrigation challenge the concept of success of hydrophobic sealers. Concomitantly these collagen matrices offer an opportunity for dentin hybridization with hydrophilic methacrylate-based resins/sealers without the adjunctive use of phosphoric acid etching or self etching primers.

**Franklin R. Tay et al.(2006)[42]** reported that with the use of 1.3% NaOCl and distilled water as initial and final rinse under mechanical agitation resulted in retention of 0.5 to 1 μm thick smear layers. The Bio pure MTAD with reduced irrigation time of 2 minutes removed smear layers completely and created 5 to 6 μm thick zones of demineralized dentin matrices on the root canal surfaces. The use of 17% EDTA for 2 minutes also resulted in complete smear layer removal and demineralization of 1 or 2 μm of intra radicular dentin. The study confirmed that the use of a 2 minute final irrigation time did not compromise the smear layer removal capability of both Biopure MTAD and EDTA. Present result were compared to study done previously using 5 minute irrigation time, thickness of the demineralized dentin was reduced from 10 -12 to 5 -6 μm for MTAD solution and 1-2μm for EDTA solution.

**Matthias Zehnder (2006)[43]** reviewed the requirement for irrigating solutions. Sodium hypochlorite due to its broad anti microbial spectrum as well as capacity to dissolve necrotic tissue remnants is recommended as main irrigant. During canal instrumentation canals should always be filled with sodium hypochlorite. This increases working time of the irrigant. The cutting efficacy of hand instruments is improved and torsional load on rotary nickel-titanium instruments is reduced when canals are filled with sodium hypochlorite solution. A 5.25% sodium hypochlorite solution significantly decreases the elastic modulus and flexural strength of human dentin compared to physiologic saline, while a 0.5% solution does not decrease the elastic modulus and flexural strength. Alternative approach to improve effectiveness of hypochlorite in root canal system could be to increase the temperature of low – concentration sodium hypochlorite solutions. 1 mol of hypochlorite contains 1 mol of available chlorine. State of available chlorine is depending on the pH of the solution. Above pH of 7.6, the predominant form is hypochlorite, below this value is hypochlorous acid. Both forms are extremely reactive oxidizing agents. Pure hypochlorite solutions used in endodontics have a pH of 12, thus entire available chlorine is in the form of hypochlorite. At identical levels of available chlorine, hypochlorous acid is more bactericidal than hypochlorite . One way to increase the efficacy of hypochlorite solution could be to lower their pH. Such solutions was found to be less toxic to vital tissues than nonbuffered solutions.Buffered hypochlorite with bicarbonate renders the solution unstable with a decreased shelf life to less than 1 week.

**Luciano Giardino et al.(2006)[44]** compared the surface tension of four common endodontic irrigants – 17% EDTA, Cetrexidin, Smear Clear, 5.25% Sodium hypochlorite with the surface tension of MTAD and Tetraclean. Results showed that MTAD had a low surface tension (34.5 $mJ/m^2$) , similar to Cetrexidin one (31.1 $mJ/m^2$). The lowest surface tension was shown by Tetraclean (29.1 $mJ/m^2$). The surface tencion vaue for Smear Clean was 33 $mJ/m^2$).

**Mercedes Perez-Heredia et al.(2006)[45]** evaluated the cleaning ability of three acid irrigating solutions after hand and rotary instrumentation. The irrigating solutions were 1) 15% citric acid plus 2.5% NaOCl 2) 15% EDTA plus 2.5% NaOCl 3) 5% orthophosphoric acid plus 2.5% NaCl and 4) 2.5% NaCl alone as control. Neither the smear layer nor debris was removed combining 15% EDTA with 2% NaOCl with both instrumentation technique in the coronal, middle and apical thirds. With hand instrumentation, 15% EDTA showed the best effectiveness in debris removal in coronal, middle and apical thirds. With regard to smear layer removal 15% EDTA showed major effectiveness in apical and middle third. With rotary instrumentation technique 15% citric acid plus 2.5% sodium hypochlorite removes smear layer in apical, middle and cervical thirds. Cervino et al. found that 17 % EDTA and 15% citric acid alternated with 5% sodium hypochlorite were equally effective in smear layer removal with the hand instrumentation. Orthophosphoric acid is a universal conditioner shown to have smear layer removal capability. Partial removal was obtained with 10 second application of 10% phosphoric acid or 10% citric acid and complete elimination achieved after similar treatment using 32% phosphoric acid. In debris removal orthophosphoric had the had the least behavior.

**Gonzalez-Lopez et al.(2006)[46]** evaluated the demineralizing capacity of 10% and 20% citric acid and 17% EDTA after 3, 10 and 15 minutes of immersion.

The effect of 1% chlorhexidine has been studied. The specimens were immersed in 25ml of each of solutions at 3, 10 and 15minutes of immersion. The concentration of $Ca^{2+}$ was measured by atomic absorption spectrometry. Results showed no significant differences in the amount of $Ca^{2+}$ extracted by 10% or 20% citric acid or by 17% EDTA. EDTA extracted a significantly higher amount during first three minutes compared with other solutions.The decalcifying action was time dependent. The amount of $Ca^{2+}$ extracted in the citric acid and EDTA solutions increased with longer immersion time. Study shows that 17 % EDTA and the two citric acid concentrations have the same decalcifying power but the effect of EDTA is more rapid. A higher amount of $Ca^{2+}$ was obtained in the first 3 minutes with 17 % EDTA than with other solutions. Application of 17 % EDTA for 10 minutes causes excessive erosion of peritubular and intertubular dentin. A short periods are recommended for its application.

**Stevens et al.(2006)[47]** conducted a study in which the maxillary incisors were decoronated, prepared in crown down fashion and smear layer removed with 17% EDTA followed by 5.25% NaOCl (Group N) and again rinsed with NaOCl before obturation and Roth 801 sealer was used. The roots in a group were rinsed with 95% ethyl alcohol instead of NaOCl as final rinse. Leakage was determined using fluid flow model. Result showed that final rinse with 95% ethyl alcohol increased sealer penetration and decreased leakage.

**Marending et al.(2007)[48]** evaluated mechanical, chemical and structural alterations of human root canal dentin following exposure to ascending concentrations of sodium hypochlorite. Results showed the permeability of altered dentin was markedly enhanced with exposure to 1% NaOCl and more with 5% NaOCl solution. A concentration dependent reduction of elastic modulus and flexural strength of root dentin resulted. 5% sodium hypochlorite severely altered the peripheral dentin matrix..Stoward and Davies reported that sodium hypochlorite fragments long peptide chains and to chlorinate protein terminal groups; the resulting N-chloramines are broken down into other species. Thus sodium hypochlorite solutions may affect mechanical dentin properties via the degradation of organic dentin components. The study showed a concentration dependent effect of sodium hypochlorite on mechanical dentin properties resulting from the disintegration of organic dentin matrix.

**N.Nisha Soumithran et al.(2007)[49]** studied the demineralizing effect on radicular dentin by 17% EDTA and MTAD at different time intervals. The assessment of amount of phosphorous liberated at different time intervals was done using random access automated bio chemistry analyzer. The results of the study concluded the use of MTAD as a chelating agent followed by a final flush with saline as irrigant is better than 17% EDTA. The irrigants have to be flushed out of the canal within 10 minutes. Care must be taken to avoid the use of MTAD in children and pregnant women for its deleterious action on dental structures.

**Sandeep Singh et al.(2007)[50]** studied the effect of EDTA, EDTAC, RC. Prep and MTAD on coronal middle and apical root canal dentin. It has been suggested that chelating agents and acids remove smear layer from root canals as the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them highly soluble in acids. The results for four irrigants showed that microhardness of root canal dentin was reduced for all. Bio Pure MTAD was least effective in reducing microhardness of root canal dentine and 17% EDTA had maximum effect. Panighi and G'Sell reported positive correlation between hardness and mineral content of the tooth. The microhardness determination provides indirect evidence of mineral loss or gain in dental hard tissues. There is a variable increase in microhardness from coronal to apical third of the root canal dentin irrespective of any treatment with test agent. This was attributed to the histology of root canal dentin. Carrigan et al. reported that tubular density decreased from cervical to apical dentin. Pashley et al. showed that an inverse correlation between dentin microhardness and tubular density.

**Sedogheh Khedmat and Noushin Shokouhinejad (2008)[51**] compared the efficacy of Smear Clear,17% EDTA and 10% citric acid in combination with 5.25% sodium hypochlorite as final irrigants in smear layer removal. Smear Clear (Sybron Endo, Orange, CA) is a product containing 17 % EDTA containing a cationic (cetrimide) and an anionic surfactant. The study showed that addition of two surfactants to EDTA (Smear Clear) did not improve its efficacy in smear layer removal compared with surfactant free-EDTA. The results showed that smear layer removal with 10% citric acid and 17% EDTA has no difference. The EDTA is an effective chelating agent and erosion of dentinal tubules occurred when applied over 1 minute and in a volume more than 1ml. To minimize destructive effects on dentin, a low volume of chelating agents for a short application time is preferred. Larger canal diameter in cervical and middle thirds exposes the dentin to a higher volume of irrigants , allowing a better flow and improving the efficiency of smear layer removal in cervical and middle thirds. Crumpton et al. reported that the smear layer was efficiently removed with a final rinse of 1 ml of 17 % EDTA for 1 minute followed by 3 ml of 5.25 % sodium hypochlorite . This study reported that this protocol was not efficient to remove completely smear layer from the apical third.

**I.M.Saleh, et al.(2008)[52]** studied the effect of smear layer on penetration of bacteria along different root canal filling materials and the presence of bacteria at the interface of dentine and sealer and sealer and core material. The results showed the bacterial penetration along root filling with Gutta percha and AH plus sealer occurred more slowly in the presence of the smear layer than in its absence. Differences in leakage among these sealers using AH plus, Apexit sealer and Real seal cones and sealer was not significant when smear layer was present. The results support the view that retaining the smear layer on the root canals may be beneficial in preventing bacterial penetration and colonization. Smear layer may also act as a filler for the sealer, reducing the contraction stress that lead to pulling out of the sealer tags from the dentinal tubules.

**Perez.Heredia et al.(2008)[53]** evaluated and compared the decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid, and 2.5% sodium hypochlorite on root canal dentin. The results showed the amount of extracted $Ca^{2+}$ increased with time in all solutions, and no significant differences between 15% EDTA and 15% citric acid. At three immersion times (5min, 5-10min, 10-15min) the decalcifying capacity of 15% EDTA and 15% citric acid solutions were higher than 5% phosphoric acid solutions. The decalcification mainly occurred during first 5min of action.

**Emboava Spano et al.(2009)[54]** evaluated the concentration of calcium ions and smear layer removal using 15% EDTA, 10% citric acid, 10% sodium citrate, apple vinegar, 5% acetic acid, 5% malic acid and sodium hypochlorite. Results showed that use of 15% EDTA resulted in greatest concentration of calcium ions, followed by 10% citric acid. 15% EDTA and 10% citric acid were the most efficient solutions for removal of smear layer. Zehnder et al. studied the effect of reducing surface tension of 15.5 % EDTA, 10% citric acid, or 18% 1-hydroxyethylidene-1, 1-bisphosphonate [HEBP] prepared with and without 1% polysorbate (Tween) 80 and 9% propylene glycol on their ability to remove calcium from instrumented root canals. The results showed no increase in calcium chelating ability when surface tension of chelator solutions was lowered with wetting agents. Citric acid removed more calcium than EDTA or HEBP solutions.

**Xin-Hua Gu et al.(2009)[55]** studied the effect of different irrigating solutions on smear layer removal and tubular opening on root canal surfaces after post space preparation. Study also evaluated if ultrasonic irrigation has any effect on smear layer removal. The solutions EDTA, sodium hypochlorite, sodium chloride were used alone and with ultrasonic activation. When fibre post and resin luting systems were used to restore endodontically treated teeth, the adhesive bonding to dentin is based on micromechanical retention created by demineralized surface and resin tag formation. EDTA and sodium hypochlorite have low surface tension , that contribute to improved wet ability and flow of the irrigating solutions. The results showed that EDTA performed significantly better than NaCl and NaOCl in smear layer removal and dentinal tubular opening. Ultrasonic activation of the irrigation could not improvr removal of smear layer and opening of dentinal tubules.

**Punit Bansal and Hitesh Gupta (2009)[4]** reviewed on smear layer in endodontics. The article explains the importance of smear layer. The presence of smear layer on root canal walls acts as an intermediate physical barrier and may interfere with adhesion and penetration of sealers into dentinal tubules. When smear layer is not removed, the durability of apical seal has to be evaluated over a long period, since this layer is non homogenous and weakly adherent structure, dissolving around a leakage filling material and thus creating a void between the root canal wall and the sealer.

**M.A.Mozayeni et al.( 2009)[5]** studied the effectiveness of MTAD as the final irrigant to remove the smear layer, compared with that of 17% EDTA, both following root canal irrigation with 5.25% sodium hypochlorite. When EDTA is alternately used with 5.25% NaOCl, the smear layer is removed in middle and coronal thirds of canal preparation, and is less effective in the apical third. In this study placement of MTAD with wrapped cotton in broach allows intimate contact of solution even in apical region of the canals and improves debridement of the entire root canal wall.

**Lottanti et al.(2009)[56]** evaluated the effects of ethylenediamine tetra acetic acid, etidronic acid and peracetic acid used in combination with sodium hypochlorite on smear layer. The study result showed that smear layer in instrumented root canals could be removed employing either etidronic or peracetic acid to a similar extent produced with conventional EDTA treatment. An etidronic acid/sodium hypochlorite mixture can be applied as a sole irrigant. Etidronic acid is biocompatible and is used as an additive in various personal care products such as soaps. It is also used in swimming pools because of its compatibility with hypochlorite to prevent stains from metal ions. Irrigation protocols using 1% sodium hypochlorite and then 17 % EDTA, 1% sodium hypochlorite and then 2.25% peracetic acid or a combined solution containing 1% sodium hypochlorite and 9% etidronic acid produced similar efficiency in smear layer removal.

**Mancini et al.(2009)[57]** compared the efficacy of 17 % EDTA, Bio Pure MTAD and 42% citric acid in endodontic smear layer removal and degree of erosion in the apical third of instrumented root canals. The study showed Bio Pure MTAD did not remove the smear layer from apical third of the canals. The study using citric acid 42% did not remove smear layer from the apical third of the canals. 5.25% Sodium hypochlorite at $37^{o}$ C did not remove smear layer from the apical third of the canals. In addition to sodium hypochlorite, the application of chelating agents to remove smear layer has been suggested. Calt and Sepper and O'Connell et al. found the combination of 17% EDTA and 5 % sodium hypochlorite is an effective irrigation solution in removing smear layer in the apical third of instrumented canals.

**Swaty Jhamb et al.(2009)[58]** compared the microleakages of Acroseal and Ketac –Endo with and without smear layer removal.17% EDTA and 17% EGTA were used for smear layer removal and smear layer retained in samples using 5 % sodium hypochlorite.The results showed microleakage highest with 5% sodium hypochlorite. The reason for this is sodium hypochlorite does not remove smear layer but only flushes out the organic debris. 17% EDTA and 17% EGTA removed smear layer completely. 17% EDTA when used as a chelator, caused softening of dentin, and enlargement of dentinal tubules. 17% EGTA removed smear layer without inducing erosive action and allowed sealers to penetrate dentinal tubules completely. The sealing ability of Ketac-Endo was higher in comparison with groups using Acroseal.

**D.R.Violich and N.P Chandler( 2010)[3]** explained the current methods to smear layer removal - chemical, ultrasonic and laser techniques and none of which are totally effective throughout the length of all canals or are universally accepted. Smear layer is removed for thorough disinfection of the root canal system and for better adaptation of materials to the canal walls. The adaptation of root canal materials to canal walls were studied. White et al. found pHEMA, silicone, Roth 801, and AH 26 sealers extended into the tubules when smear layer was removed.

**Vishal A.Mahajan et al.(2010)[59]** evaluated the effect of MTAD and EDTA in removing the smear layer and its effect on peritubular and intertubular structures using SEM examination. The results indicated that MTAD is an efficient solution for removal of smear layer, especially in the apical third of root canals, and does not cause structural change in dentinal tubules. The smear layer removal action of EDTA is attributed to chelation action. The moderate smear layer removal by EDTA in the apical third of root canal attributed to poor penetration of EDTA in the apical area of root canal. When comparing the degree of erosion from cervical and middle third areas in MTAD and EDTA treated groups , a statistically significant difference was found. MTAD group showing no erosion. Erosion of dentinal tubules can be due to hyperdecalcification induced by EDTA on dentin.

**Caron et al.(2010)[60]** examined the effect of different final irrigation regimens on smear layer removal in curved canals after root canal instrumentation.

The study compares the effect of mechanical agitation using a fully tapered and apically trimmed non standardized gutta percha master cone, Rins endo irrigation system and Endoactivator system in removing the smear layer. The study found activations resulted in cleaner canals compared with no activation. In this study the access cavity provided a strategic reservoir to hold a more effective volume of irrigant for exchange during activation. The irrigating solutions are apically exchanged each time the activator system is inserted into the canal. When the activator tip moves toward length, reagent id displaced. When activator is partially withdrawn there is exchange of solution into the apical third. Efficiency of this hydrodynamic circuit is further enhanced when combined with sonic oscillating movements. A pumping action synergistically combined with mechanical agitation explains better results achieved with the EndoActivator.

**R.Rajasingham et al.(2010)[61]** evaluated the effect of irrigation regimens on tooth surface strain using saline, sodium hypochlorite (3% and 5% NaOCl) and ethylene diamine tetra acetic acid (17% EDTA) were evaluated individually and as alternating combinations. The results showed alternate irrigation with 5% NaOCl and 17% EDTA resulted in increase in tooth surface strain. The teeth irrigated with 17% EDTA shows negligible change in strain. Alternate irrigation with 3% NaOCl and 17% EDTA produced noticeable increase in surface strains. The result stresses the need to select a suitable lower concentration of NaOCl that would have minimal undesirable effects on physical properties of dentin. Alternative irrigation with 5% sodium hypochlorite and 17 % EDTA resulted in tooth surface strain that were highly significant from irrigation with saline.

**Xiaoli Hu et al. (2010) [62]** evaluated the effect of 17% EDTA, 5.25% NaOCl, 3% $H_2O_2$ on dentin wettability and roughness . The wettability is a crucial factor for adhesion and is dependent on chemical composition, roughness and hydration state and is influenced by tubule density. The study found 5.25% NaOCl increased the wettability of dentin. The roughness of dentin increased with NaOCl application because of organic dissolving properties of NaOCl on collagen components. The dissolution of inorganic components by EDTA by its chelating action softens root canal wall and tubules become patent, surface roughness is increased due to smear layer removal. The chelating action of EDTA softens root canal wall by dissolution of inorganic components. Dentin tubules become patent and surface roughness increased.

**Rodig et al(2010)[63]** evaluated the cleaning efficacy of different irrigant agitation techniques on debris and smear layer removal in curved root canals. The effectiveness of ultrasonic activation, endo activator, and canal brush was evaluated. The agitation devices were introduced 2mm short of working length, activation time for each irrigant was 1 minute. The results showed that endo activator was superior in smear layer removal compared with ultrasonic agitation and canal brush. The removal of smear layer was more effective in coronal than in apical region. The activation of irrigant improved smear layer removal, but no benefit of irrigant activation was observed in apical portions.

**Nidambur Vasudev Ballal et al.(2010) [64]** evaluated the effect of 7% maleic acid and 17% EDTA solutions on the microhardness and surface roughness of root canal dentin. The result showed no statistical difference between 7% maleic acid and 17% EDTA, and found 7% maleic acid significantly better than EDTA in removing smear layer from the apical third of root canal system. Microhardness determination provide indirect evidence of mineral loss or gain in dental hard tissues. Changes in mineral content may adversely affect the sealing ability and adhesion of dental materials such as resin-based cements and root canal sealers. The degree of mineral content and amount of hydroxyapatite in intertubular substance are factors determining the intrinsic hardness of dentin structure. 7% maleic acid is highly acidic and a pH of 1.05. This acidic pH caused demineralization of dentin and reduction in microhardness. There was no statistically significant difference between 7% maleic acid and 17% EDTA.

**De Deus et al.(2011)[65]** evaluated the effect of the exposure time and concentration of peracetic acid (PAA) on removal of smear layer. The study showed that concentration of 2.25% PAA can dissolve an experimental smear layer as quickly as a standard 17% EDTA solution. After 60s of contact, the 0.5% peracetic acid solution dissolved smear layer as well as 2.25% PAA and 17% EDTA. 17% EDTA, 2.25 % peracetic acid and 9% etidronic acid removed smear layer and demineralized the root canal wall. After 60 seconds of contact, the 0.5% peracetic acid solution dissolved smear layer as well as 2.25% peracetic acid and 17% EDTA.

**Adiguzel et al.(2011) [66]** stated that the use of 17% EDTA and MTAD with continuous irrigation using SAF resulted in efficient debridement at all thirds of root canal system. A 2 minutes continuous irrigation using MTAD resulted in percentage of smear layer removal in coronal middle and apical third of 85%, 70% and 60%. And for debris this was 95%, 90%, 95% while with EDTA resulted in percentage of smear layer removal of 85%, 60% and 50% and of debris was 95%, 90%, 85%. SAF a newly developed file system, when inserted into a root canal adapts itself to the canal's original shape. The surface of the lattice is slightly abrasive, and the system removes dentin with a back-and-forth grinding motion with vibration. An irrigation device (Vatea) is connected to the silicon tube and provides continuous flow of the preferred irrigation solution. This motion creates turbulence in the root canal allowing continuous and fresh irrigant present in the canal. The use of the SAF in combination with a dual-irrigation regimen of 3% sodium hypochlorite and 17% EDTA has been reported to create in clean dentin surface especially in the apical third. Successful removal of smear layer for both EDTA and MTAD from the entire root may be due to vibrating motion of SAF within the continuously replaced fluid. SAF system vibrating at 5000 vibration /minute induces sonic activation of the chosen irrigant.

**Patil and Uppin (2011)[9]** evaluated the effect of widely used irrigating solutions on root dentin microhardness and surface roughness. The results of the study indicate all irrigating solutions except chlorhexidine decreased microhardness of root canal significantly and 3% $H_2O_2$ and 0.2% chlorhexidine gluconate had no effect on surface roughness. A significant increase on surface roughness was found in 2.5%, 5% NaOCl and 17% EDTA. Hulsmann et al. reviewed the mode of action of EDTA. Properties of EDTA were self-limiting. EDTA with neutral pH showed chemically two co-existing reactions (i) complex formation (ii) protonation. The exchange of calcium from the dentin by hydrogen resulted in a decrease in pH. Because of release of acid, efficiency of EDTA decreased with time. The observations suggest that canal irrigation with chemical solutions results in structural changes, as shown by reduction of dentin microhardness and increase in surface roughness. This effect were related to the demineralizing effect on root canal dentin.

**Prado et al.(2011)[67]** compared the effectiveness of 37% phosphoric acid with that of 17% EDTA and 10% citric acid in the removal of smear layer. The results showed that at 3 minutes of application, phosphoric acid solution was more effective, in the apical third, followed by citric acid, EDTA and phosphoric acid gel. When comparing the solutions in cervical, middle, and apical thirds, EDTA and citric acid were more effective in cervical third than middle and apical thirds. Phosphoric acid solution was equally effective in cervical, middle and apical thirds. Phosphoric acid gel was more efficient in the cervical and middle thirds than in the apical third. 37% phosphoric acid showed dentin erosion related to time of exposure. At 1 minute or longer, the erosion was present in middle and cervical thirds and no erosion found in the apical third.

**Rossi-Fedele et al.(2011)[68]** reviewed the influence of $p^H$ changes on the efficacy of chlorine containing endodontic solutions. $P^H$ value of sodium hypochlorite between 6 and 7.5 would lead to improved antibacterial efficacy. The tissue dissolution activity of sodium hypochlorite decreased when $p^H$ reached values between 6 and 7.5. Zehnder explained the property of sodium hypochlorite used widely as main root canal irrigant because of its broad antibacterial activity, its function to prevent formation and to dissolve the smear layer and its ability to dissolve tissue remnants. Chlorine has a strong tendency to acquire electrons in order to achieve greater stability, and this translates into chlorine's oxidizing activity. Oxidizing capacity is retained by hypochlorous acid (HOCl) , its hydrolysis product. This hypochlorous acid is responsible for the disinfectant action of chlorine solutions. The relative amount of hypochlorite ion and HOCl present in chlorine solutions at a given $p^H$ and temperature is constant, as HOCl in water undergoes an instantaneous and reversible ionization into hypochlorite ($OCl^-$) and hydrogen ($H^+$) ions. Subsequently, $p^H$ changes will reflect the relative amounts of hypochlorite ion and HOCl present in the solution. If HOCl is consumed, then the balance will shift, new HOCl will form at the expense of $OCl^-$. The $OCl^-$ in the aqueous solution can work as reservoir for the formation of new HOCl and vice versa. Lowering the $p^H$ to values below 4 and 5 diminishes the relative amount of HOCl and chlorine gas ($Cl_2$) dissolved in water increases at the same rate. Chlorine gas form is unstable because of volatility and has noxious odour and irritant to the respiratory tract, eyes, and mucous membrane and at higher concentrations can have fatal effects.

**Ametrano et al.(2011)[69]** evaluated the effects of sodium hypochlorite and ethylenediaminetetraacetic acid on rotary nickel- titanium instruments using atomic force microscopy. Nickel –titanium instruments became popular because of superior elasticity and resistance to torsional fracture compared to stainless steel hand files. Studies reported that NiTi instruments may be used up to ten times in simulated canals, can prepare four molar teeth without fracture. Along with cleaning and sterilization procedures the contact with irrigating solutions including sodium hypochlorite and ethylenediaminetetraaceic acid can enhance their corrosion and deterioration and lead to fracture. Atomic force microscopy has become increasingly popular for imaging the three-dimensional surfaces and interfaces of biomaterials. Atomic force microscopy has also been recommended as a valuable research tool for investigating the topography of various endodontic instruments. AFM is part of the scanning probe microscopy family and is possible to reconstruct three-dimensional surface topography images in real time. Topographic surface changes in ProTaper instruments immersed in sodium hypochlorite and ethylenediaminetetraacetic acid solutions were evaluated. Significant deterioration of instrument surfaces resulting in an increase in root mean square value and roughness average were caused by both irrigants. Localized pitting and cracks that modifies the integrity and resistance to fracture of NiTi instruments were seen on immersion in 5.25 % sodium hypochlorite for 5 minutes.

**De-Deus et al.(2011)[70]** made critical appraisal of published smear layer removal studies. In 2007 De-Deus et al. successfully developed an optical microscopy method, called cosite optical microscopy (CSOM) that allows longitudinal observation of a predetermined dentin area. CSOM produces a set of images from a large number of x-y positions of dentin sample at different experimental times. Different from the traditional score analysis, CSOM provides operator-independent quantitative results with more reliable statistics for comparison. For each sample, at each experimental time, the 15 analysed image fields contained between 6000 and 7000 tubules. For each substance evaluated, approximately 20,000 tubules were automatically measured at each experimental time without operator influence. The procedure is fast taking about 25 minutes for image acquisition for all experimental times, and 3 minutes for image analysis of the full set of images using a common personal computer.

**Stojicic et al.(2012)[71]** studied the antibacterial and smear layer removal ability of novel irrigant solution Q-Mix with MTAD solution and EDTA . The study reported that Q Mix has better antibacterial property and contains chlorhexidine and EDTA and that smear layer removing ability was comparable to EDTA solution. Irrigants play an important role in accessing areas not instrumented such as lateral and accessory canals as well as fins and webs throughout the canal. None of present irrigants meets all requirements of an ideal endodontic irrigant. Sodium hypochlorite in concentrations from 0.5 % to 6 % is the most accepted irrigating solution. Sodium hypochlorite has antibacterial, tissue dissolving properties. It is toxic to periapical tissue and suggested to decrease micromechanical characteristics of dentin. Has no action on inorganic part of smear layer and EDTA used in 17 % concentration to dissolve inorganic portion of dentin and smear layer by chelation. EDTA is recommended for use after sodium hypochlorite to remove smear layer. Quin et al reported that if NaOCl is used again after EDTA or citric acid as final antibacterial rinse , it cause marked erosion of root canal dentin. Considerable efforts made on developing new irrigants and/or establishing new irrigation protocols. Bio Pure MTAD were introduced in 2003. It was suggested to be more effective than NaOCl and EDTA against Enterococcus faecalis and mixed bacteria. QMiX is a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX and a detergent, It is a clear solution ready to use with no chair side mixing. Mixing EDTA and CHX is known to produce a white precipitate. In QMiX this is avoided because of its chemical design.

**Lofti et al.(2012)[7]** evaluated the effect of MTAD as a final rinse on removal of smear layer subsequent to primary irrigation with 1.3% sodium hypochlorite during 10 minute instrumentation. At present, rotary instruments can shape a canal in less than 10 minutes and the contact time of sodium hypochlorite with canal walls as initial rinse is significantly reduced. The MTAD protocol for irrigation is using 1.3% sodium hypochlorite as initial rinse followed by MTAD solution as final rinse, Torabinejad studied the effect of MTAD by using 1.3 % sodium hypochlorite as initial rinse for a cumulative period of 18 -20 minutes during instrumentation and left MTAD solution for 2 minutes after instrumentation . Smear layer removal was obtained using the above irrigation protocol. Lofti et al. evaluated the effect of MTAD on removal of smear layer as final rinse when 1.3% sodium hypochlorite was used during 10 – minute instrumentation time. In the study MTAD could not eliminate the smear layer from root canal walls. It was concluded that the irrigation with 1.3% NaOCl during instrumentation for 10 minutes was not effective to remove organic materials from the smear layer and consequently MTAD could not remove smear layer effectively.

**Palazzi et al.(2012)[72]** compared the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite – based endodontic irrigants. Chlor- Xtra, Hypoclean A and Hypoclean B. High surface tension could affect the ability of sodium hypochlorite to penetrate dentin and reducing its antibacterial effectiveness. For obtaining a suitable contact time of NaOCl with dentin walls , a major factor is the wettability of the the irrigant on dentin, Wettability is correlated with surface tension. To achieve this optimal wettability the surface energy of the substrate must be as high as possible and surface tension of the liquid contacting the substrate must be as low as possible. The surface tension of 5.25% NaOCl is 48.90 $mJm^{-2}$ . According to manufactures, Hypoclean A and B are NaOCl solutions containing 5.25 % NaOCl and two different surfactants: cetrimide (cationic surfactant) and polypropylene glycol (PPG). Surface tension of Hypoclean A solution was 29.13 mJ $m^{-2}$ significantly lower than 5.25% NaOCl. Hypoclean A demonstrated a more effective antibacterial action against E.faecalis than 5.25% NaOCl. It can be assumed that low surface tension increases NaOCl penetration into inaccessible areas of the root canal system and dentinal tubules.

# MATERIALS AND METHODS

## Materials used in the study –

a) 5.25 % Sodium hypochlorite ( Vensons India)

b) 17 % EDTA ( Himedia Laboratories)

c) 17% EGTA (Himedia Laboratories)

d) 10% Citric acid (Spectrum Reagents and Chemicals Pvt Ltd)

e) BioPure MTAD ( Dentsply, Tulsa dental specialities)

f) Distilled water

g) Paper Points ( Suredent Corporation)

h) 7% Glutardehyde ( SDFCI Fine Chem Ltd)

i) Ethanol

j) Self cure acrylic resin (DPI)

k) Plastic ring

l) Silicon carbide polishing paper

## Equipment used in this study –

a) 10 number K –File ( Mani)

b) 28 gauge side vented irrigation needle

c) X Smart Endo rotary system ( Dentsply)

d) RaCe File system ( FKG Dentaire)

e) Micromotor and diamond disc

f) Chisel and mallet

g) JEOL JSM-5600LV Scanning electron microscope

h) Vickers microhardness testing machine ( CLEMEX)

## Methodology -

Sixty non-carious freshly extracted mandibular pre-molars for orthodontic reasons were used in this study. After cleaning they were immersed in isotonic saline solution. Then the teeth were decoronated and a 10 number K- file was inserted into the canal until just visible at apex to determine patency. One millimeter was subtracted from this measurement and this was the working length.

The teeth were divided randomly into five experimental groups of 12 each.

Group I – 10 ml of 17 % EDTA was used as a final rinse for 5 minutes

Group II – 10 ml of 17% EGTA was used as a final rinse for 5 minutes

Group III – 10 ml of MTAD was used as a final rinse for 5 minutes

Group IV – 10 ml of 10% citric acid was used as a final rinse for 5 minutes

Group V – 10 ml of 17 % and 5.25 % sodium hypochlorite. (fig. 2)

While instrumentation, 5.25 % sodium hypochlorite was used as the main irrigant. All teeth were prepared by rotary instrumentation using RaCe to an apical size of ISO 30 / 0.06. After instrumentation each canal was irrigated with 10 ml distilled water and dried with paper points. A total of 10 ml irrigant at the rate of 1ml per 30 sec was used as final irrigant for each root canal.

The total exposure time to final solution was approximately 5 minutes. Irrigants were passively delivered using side vented 28 guage needle to within 1-2 mm from the working length in each canal. The specimens were fixed using glutaraldehyde and the fixed specimens were rinsed three times with a sodium cacodylate – buffered solution (Concentration 0.1, pH 7.2), incubated in osmium tetraoxide for 2 hours, dehydrated with ascending concentrations of ethyl alcohol and placed in a desiccator for 24 hours. The specimens were split into two halves using chisel and mallet along the prepared groove (fig.3).Each specimen was mounted on a aluminum stub and coated with 30 μm of gold palladium and examined under scanning electron microscope (fig.4).The smear layer on the surface of the root canal or in the dentinal tubules at the cervical, middle and the apical portion of each canal was evaluated according to the following criteria used by Torabinejad et al.

1- No smear layer: no smear layer on the surface of the root canals, all tubules were clean and open.

2- Moderate smear layer: no smear layer on the surface of the root canal, but tubules contained debris.

3- Heavy smear layer: smear layer covered the root canal surface and the tubules.[34]

The other half of longitudinally split portions were ground polished with water cooled carborandum disc. Final polishing was carried out in felt cloth and buff using

0.05 µm size aluminum oxide powder mixed with distilled water.A plastic ring was then taken and poured with a mixture of cold cure resin. Specimens were embedded on the resin with polished surface facing outside. After curing of the resin, the ring was removed and repolishing of specimens was done to remove excess material present on the tooth surface (fig.5).Microhardness testing was done by mounting specimens on the stage of Vickers microhardness tester (fig.6). The midroot portion halfway from the outer surface was focused for testing. Indentations were made with Vickers diamond indenter using 300 gm load with a dwell time of 15 seconds. These indentations were measured and converted into Vickers hardness number (VHN).

The values obtained were statistically analyzed.

# RESULTS AND OBSERVATIONS

**Removal of smear layer** –

**Table – I** shows the smear layer scores and microhardness values obtained after final rinse with 17% EDTA

**Table – II** shows the smear layer scores and microhardness values obtained after final rinse with 17% EGTA

**Table – III** shows the smear layer scores and microhardness values obtained after final rinse with MTAD solution

**Table – IV** shows the smear layer scores and microhardness values obtained after final rinse with 10% Citric acid

**Table – V** shows the smear layer scores and microhardness values obtained after final rinse with alternating use of 5.25% Sodium Hypochlorite and 17% EDTA

**Table – VI** shows the data obtained for smear layer removal were discrete variables in scores, hence single comparisons taking groups together was performed with Kruskal – Wallis one way analysis for 5 groups and Chi square values were obtained

Mean, Median and standard deviation values for smear layer removal from cervical middle and apical portions for five groups were obtained.

Multiple comparisons of two groups taken at a time were performed using Mann – Whitney U Test and p values for all comparisons were fixed as less than 5 %.

**Table VII** shows the group comparison for cervical portions of root canal treated by comparing two groups taken at a time.

No significant difference was shown between 17 % EDTA and 10 % citric acid in cervical portions with p value > 0.05 (0.070).

When comparing 10 % citric acid with 17 % EGTA, it showed highly significant value p value < 0.05 ( p value 0.0001) . 10 % citric acid effectively removes smear layer from cervical portion than 17 % EGTA.

10 % citric acid showed highly significant value with MTAD. Also citric acid was more effective than MTAD in cervical portion of root canal. (p value 0.0001)

A statistically significant difference between 10% citric acid and combined use of 5.25 % sodium hypochlorite and 17 % EDTA was found in cervical portion with a p value < 0.05 (0.014)

**Table VIII** shows the group comparison for middle portions of root canal treated by comparing two groups taken at a time.

17 % EDTA was found to have no statistically significant difference with 10 % citric acid in middle portion of root canal (p value > 0.05, ( 0.105)) . 17 % EDTA was found to be highly significant when compared with 17 % EGTA and MTAD in middle portion (p value < 0.05 ((0.008)). 10 % citric acid also shows statistically significant difference to MTAD and 17 % EGTA in middle portion (p value < 0.05 (0.001)) . 10 % citric acid shows statistically significant difference to 5.25 % sodium hypochlorite and EDTA (p value < 0.05 ( 0.001)).

**Table IX** shows group comparison for apical portion of root canal by comparing two groups taken at a time.

10 % citric acid shows statistically significant difference between 17 % EGTA and MTAD at apical portion of root canal (p value < 0.05 (0.0001)). 10 % citric acid shows statistically significance with combined use of 5.25 % sodium hypochlorite and 17 % EDTA at apical portion (p value = 0.005 ( 0.005)).

The 17 % EDTA showed statistically significant difference between 17 % EGTA and MTAD in apical portion (p value < 0.05 (0.039)). 17 % EDTA showed highly significant difference between 10 % citric acid in apical portion ( p value < 0.05 p value (0.008)).

**Microhardness Test-**

**Table –X** shows the data obtained were statistically analyzed using parametric one way analysis of variance ANOVA and significance was tested using f test. The p value was fixed at 5 % .

The mean, median and standard deviation values for five groups were obtained .

**Table –XI** shows the multiple comparison of microhardness values between groups were evaluated using Post hoc tuckey analysis.

10 % citric acid showed statistically high significance in reduction in microhardness with 5.25% sodium hypochlorite and 17 % EDTA indicating (p value < 0.05 (0.0001)).

10 % citric acid showed statistically significant difference in microhardness with 17 % EDTA (p value < 0.05 (0.0001))

17 % EGTA and 5.25 5% sodium hypochlorite combined with 17 % EDTA showed statistically significant difference between 17 % EDTA, 10 % citric acid and MTAD.

**Table – XII** shows mean and standard deviation microhardness values for 17% EDTA, 17% EGTA, MTAD, 10% citric acid and alternating use of 5.25% sodium hypochlorite and 17% EDTA.

## Observations –

**Smear layer removal - Cervical third- ( Fig.7, Fig.10, Fig.13, Fig.16, Fig.19)**

17 % EDTA and 10 % citric acid showed no difference in smear layer property at cervical third.

10 % citric acid was more effective than 17 % EGTA and MTAD in cervical third.

A statistically significant difference p value 0.014 was found between 10 % citric acid and alternating use of 5.25 % sodium hypochlorite and 17 % EDTA.

**Smear layer removal -Middle third - ( Fig.8, Fig.11, Fig. 14, Fig. 17, Fig.20 )**

17% EDTA showed better smear layer removal property compared to 17 % EGTA and MTAD.

10 % citric acid also showed better smear layer ability compared to 17 % EGTA and MTAD. 10% citric acid was more effective than alternating use of 5.25% sodium hypochlorite and 17 % EDTA. No significant differences in smear layer removal property was observed between 17%EDTA and 10 % citric acid. No significant difference between 17 % EGTA and MTAD was observed.

**Smear layer removal – Apical third - (Fig 9, Fig. 12, Fig. 15, Fig. 18, Fig.21)**

10% citric acid was more effective than 17 % EDTA.

10 % citric acid showed p value = 0.05 when compared to 5.25% sodium hypochlorite and 17 % EDTA indicating significance in smear layer property.

MTAD showed no difference in smear layer property compared to 17 % EGTA. 10% citric acid showed more effective in removing smear layer than 17 % EGTA and MTAD.

17 % EDTA showed significant difference and better ability in removing smear layer than 17% EGTA and MTAD.

**Microhardness – (Fig. 22)**

The mean value for microhardness was found higher for EGTA group (72.6 KHV) and also for 5.25 % sodium hypochlorite and 17 % EDTA group (72.02 KHV).

10% citric acid showed more reduction in microhardness . 17 % EDTA also showed more reduction in microhardness compared to 17 % EGTA and 5.25% sodium hypochlorite and 17% EDTA.

# DISCUSSION

Smear layer is a non homogenous and weak structure adherent to tooth. This layer can harbor bacteria, is permeable to bacterial products and provide a reservoir of irritants to periradicular tissues. If smear layer was capable of favoring growth and survival of entrapped bacteria, its removal could aid in successful canal disinfection. The smear layer also interferes with the adhesion of sealer against canal walls and prevent tubular penetration of sealer, thus resulting in increased chances for leakage. It has been shown that the maximum leakage occurs between the root canal sealer and wall of the root canal. These reasons favors the need for removal of smear layer prior to obturation.Whether the smear layer should be removed or not still remains a controversy. If smear layer acts as a substrate for growth and survival of bacteria and alter dentin permeability leading to ineffective disinfection of dentinal tubules, then its removal contributes to successful root canal treatment.[35]

The scanning electron microscopy shows that the smear layer contains both organic and inorganic substances. The smear layer consists mostly of inorganic substances and root canal irrigation with sodium hypochlorite has little effect on removal of smear layer. Partial removal and not complete removal of smear layer is obtained with use of acids and chelators . The smear layer components are small particles with large surface/mass ratio, these particles are highly soluble in acid [6].

Smear layer is removed easier from cervical portion of instrumented root canals than from apical portion. Chemomechanical preparation provides debridement of root canal system. This is achieved using instrumentation combined with effective irrigation with solutions.This procedure enables to prepare a clean, debris free canal for subsequent obturation.[9]

Sixty single rooted noncarious mandibular pre molars used in this study were sectioned near cementoenamel junction before preparation to standardize the root length. 5.25 % sodium hypochlorite was used as initial irrigant in all the samples which were instrumented using rotary crown down technique with RaCe rotary files. The apical enlargement were standardized to 30 size 0.06 taper file.

The literature shows numerous variety of irrigation time and quantity of irrigants in removing smear layer. Historically numerous compounds in aqueous solutions have been suggested as irrigants, like the inert substances such as sodium chloride (saline) or highly toxic and allergenic biocides such as formaldehyde.

An ideal root canal irrigant

1. Should have broad antimicrobial spectrum and high efficacy against anaerobic and facultative organized microorganisms in biofilms.
2. Dissolve necrotic pulp tissue remnants
3. Inactivate endotoxin
4. Prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed.[43]

Chelating agents act on calcified tissue by substituting sodium ions which combine with dentin to give soluble salts for the calcium ions that are bound in a less soluble combination. The most popular of the chelating agents is EDTA introduced by Nygard Ostby (1957). EDTA is a liquid solution of the sodium salt of Ethylene diamine tetraacetic acid with a pH of 7.3. In a normal concentration it removes 10.6 g of calcium from100g of calcium. Although introduced into endodontics with the aim to facilitate preparation of calcified and narrow root canals by softening root canal dentine, it also has been suggested as a useful irrigant owing to its capacity to remove the smear layer. Vander Fehr and Nygard Ostby presented a combination of Cetavalon and EDTA, this modification has been known commercially as EDTAC. The action of this solution has been described to be self limiting and not exceeding a depth of 50 micro meter after 48 hour exposure. The use of EDTA as an antibacterial agent and intratubular disinfection action is limited. The use of EDTA in vitro has shown to inhibit the substrate adherence capacity of macrophages when leaking into the peri apical tissues, thus reducing both peri apical inflammatory reactions and peri apical healing. In1969 Stewart added ureaperoxide to EDTA. In contact with sodium hypochlorite this creates foam which lifts debris out of the root canal. A commercial product using this mixture has been known as RC – Prep. The addition of surfactants and disinfectants lowers the surface tension of EDTA and enhances the removal of superficial smear layer so that the antiseptic components can penetrate the dentinal tubules This product has been marketed as Tublicid.[31]

The action of EDTA is self limiting and the demineralization stops when an equilibrium between calcium ion in dentin and the chelating agent has been reached. The cleansing ability of EDTA seems better in the middle and coronal portion of the root canal. The effect has been shown to be confined to prepared root canal surfaces whereas no effect on organic tissues in instrumented root canal could be detected. The use of sequential irrigation of EDTA and Sodium hypochlorite has been shown to be more effective in smear layer removal than use of two solutions alone as well as in antibacterial efficacy. The combined use of EDTA and ultrasound did not enhance the dissolving capability of EDTA [31].

The disodium salt of EDTA at 17 % concentration and neutral pH is widely preferred for root canal treatment. The efficiency of EDTA solution on the demineralization of dentin is influenced by pH and the greatest demineralization efficiency of EDTA solutions (0.3M) can be achieved between pH 5.0 – 6.0. On the other hand, commercially available preparations of EDTA have an average pH of 7.3. The combination regimen of sodium hypochlorite and EDTA produced less erosion and effectively removed smear layer from cervical, middle and apical portions.[28]

Since single rooted mandibular premolars without curvature and no complexity of root canal anatomy were selected.The tooth samples were sectioned longitudinally for evaluation of smear layer removal from cervical, middle and apical portion. [26]

EDTA remains the proven effective chelating agent for removal of smear layer. The study on time dependent effects of EDTA on dentin structures by Calt S et al. reveals the erosion of dentinal tubules caused by application of EDTA over 1 minute and in a volume more than 1 ml. Crumpton et al. showed that the smear layer can be efficiently removed with a final rinse of 1 ml of 17 % EDTA followed by 3 ml of 5.25% sodium hypochlorite.In this study EDTA caused erosion of dentin structures in coronal and middle third, and was comparatively not effective in removal of smear layer from apical third as shown by Crumpton et al.[51]

Murray et al. also showed that using 17 % EDTA was not efficient in removing smear layer from all the instrumented root canals. A larger diameter of root canals in coronal and middle third exposes dentin to a higher volume of solution and allows better flow of the solution resulting in better removal of smear layer in coronal and middle third of root canal as compared to apical third.[38]

In this study the final rinse with 10 ml of 17 % EDTA for 5 minutes removed smear layer from cervical and middle portions of the instrumented root canals and caused erosion of intertubular dentin from cervical, middle and apical portions of the root canal. ( Fig.12) Application of 17 % EDTA caused erosion of intertubular and peritubular dentin in coronal middle and apical portion of root canal. In this study 10 ml of 17 % EDTA solution application for 5 minutes using rotary instrumentation created a complete removal of smear layer from mostly coronal, middle and to a less extent in apical regions of root canals.

EDTA acts by dissolving inorganic component of the smear layer and acts as a chelator reacting with calcium ions in hydroxyapatite crystals, removing calcium ions from the dentin. Efficacy depends on factors like penetration depth of the material, hardness of the dentin, duration of application, the pH, and the concentration. EDTA when used for prolonged duration caused dissolution of dentin at the expense of peritubular and intertubular dentin. Chelating action of EDTA was more prominent during first few minutes .[27]

Chelation efficacy of EDTA depends on ratio of ionized to non ionized molecules in the solution. At high pH values, the excess number of hydroxyl groups will slow down the dissociation of hydroxyapatite , and limiting the number of calcium ions available. At low or neutral pH , the binding of calcium ions will tend to increase the dissociation of hydroxyapatite and its availability for chelation. [28]

The study by Serper and Calt 2002 also implies the erosive effects of EDTA solution during prolonged cleaning and shaping of root canals. Therefore lower concentration of EDTA should be preferred at neutral pH. [26].

The alternating use of 5.25% sodium hypochlorite and 5ml of 17% EDTA for 5 minutes resulted in effective smear layer removal from cervical and middle portions of instrumented root canals and more effective removal from apical portion compared to MTAD and 17% EGTA. The alternating use of 5.25 % sodium hypochlorite and 17% EDTA showed less dentinal demineralization from apical portion of root canal. (fig. 21)

EDTA retained its ability to chelate calcium in the presence of sodium hypochlorite, and tissue dissolution property of sodium hypochlorite was reduced.[28]

Alternating irrigation regimen with sodium hypochlorite and EDTA found statistically less significant p value of 0.005 when compared to 10 % citric acid in removing smear layer from apical portion of instrumented root canal walls.

The use of 10 % Citric acid effectively removed the smear layer when the canals were irrigated for 5 minutes (fig.7).

In a study by Sedigheh Khedmat and Noushin Shokouhinejad , there was only minor difference or no differences in smear layer removal with citric acid and EDTA. This may be related to use of 1 ml of Citric acid for 1 minute only. According to this study the significant difference seen in apical, middle and coronal thirds of the root canals treated with 10 % Citric acid may be related to the volume and/or application time of Citric acid ( fig. 9) .The application of higher volumes of Citric acid over 1 minute improves its efficacy in smear layer removal. [51]

In a study by A. Khademi and M. Feizianfard , 17 % EDTA produced more smear layer removal than 7% citric acid. The difference between these two materials was statistically significant. From the clinical stand point both of them seem to be acceptable. Yamada et al. reported that 17% EDTA more effective in smear layer removal than 25% citric acid. Takeda et al. found no differences between 17% EDTA and 6 % citric acid. The canal preparation was hand instrumentation and four grade scale (0-3) was to evaluate photomicrographs. Scelza et al. reported no differences between EDTA –T (a combination of 17 % EDTA and tergentol) and 10% citric acid. The study was done on straight single rooted teeth and the canals were prepared by hand instrumentation. Lenarda et al. made a comparison between 1mol/L citric acid and 15% EDTA. They prepared some samples by hand instrumentation and others by rotary profile system.They found that citric acid was more effective than EDTA, especially in samples that were prepared with profile rotary system. EDTA was more effective in samples that were prepared by hand instrumentation. Their study showed that instrumentation method could change the ability of smear layer removal with different irrigation solutions.[36]

In this study 10 % citric acid was more effective than 17 % EDTA in removing smear layer in middle and apical third of instrumented root canals ( fig.8 and fig.9). The 17 % EDTA was more effective in the coronal and middle third of instrumented root canal ( fig. 10 and fig. 11) and found less effective in the apical portion of the root canal ( fig. 12). With application of 10 ml of 17 % EDTA for 5 minutes, smear layer was removed partially from apical third when compared with 10% citric acid. 10% citric acid solution has capability of calcium extraction from a resin matrix. Citric acid has low pH and irrigation of canals with NaOCl and citric acid causes neutralization of previous irrigant.[17] Decalcification may be higher at specific pH of citric acid solution. [53]

The use of 10 ml of 10% citric acid for 5 minutes was equally effective in removing smear layer from coronal, middle and apical portions. When compared to 17 % EDTA solution, demineralization of apical portion of root canal dentin was found more with usage of 10% Citric acid ( fig.9). The efficacy of 10 % citric acid is found to be greater than with 17 % EDTA solution. The efficacy of 10% citric acid in removing smear layer from apical third of root canal when compared to 17 % EDTA was more as indicated in statistically significant p value $< 5$ (0.008).

Biopure MTAD introduced by Torabinejad et al. in 2003 is a mixture of antibiotic (Doxycycline hydrate 150mg/5ml (3%), citric acid (4.25%) and detergent (0.5% Polysorbate 80 detergent or Tween80). It has a pH of 2.15 and the recommended final irrigation for Biopure MTAD is 5 minutes. The irrigation regimen for Biopure MTAD is an initial rinse with 1.3% sodium hypochlorite followed by 5 minute final rinse with MTAD.Torabinejad et al. showed that use of 2 minutes of MTAD as final irrigant did not compromise the smear layer ability of MTAD. [33]

Torabinejad et al. reported that MTAD removed smear layer effectively and at the same time was milder on dentin structure. Giardino et al. reported that reducing the surface tension of the irrigating solution by addition of detergent can help the penetration of antibiotics into the dentinal tubules opened by citric acid. [45]

The smear layer removal property of MTAD solution was found at the cervical, middle third of root canals ( fig. 16 and fig 17), whereas at apical portion of root canal the smear layer removal was found to be less. (fig. 18) .The amount of appreciable quality of smear layer removal were less when compared with 17 % EDTA and 10 % citric acid solution. The dentinal tubular opening were uniformly same throughout the root canal portions from cervical to apical portions (fig.16 , fig 17 and fig.18). Biopure MTAD was introduced in endodontics in 2003. It was suggested to be more effective than sodium hypochlorite and EDTA against Enterococcus faecalis . Another study reported MTAD as less cytotoxic than calcium hydroxide, eugenol, 5.25% sodium hypochlorite and EDTA [71].

Dunavant et al. and Ruff et al. found the antibacterial effect of MTAD inferior to 6 % sodium hypochlorite and 2 % chlorhexidine. The MTAD protocol for irrigation is using 1.3 % sodium hypochlorite during instrumentation and MTAD solution was used as final rinse to remove smear layer. In the study by Torabinejad et al. the 1.3 % sodium hypochlorite as initial rinse were used for a cumulative period of 18-20 minutes during instrumentation and the irrigant was allowed to remain in the canal for 2 minutes after instrumentation.[7]

The effectiveness and advantage of MTAD solution appears in its ability to remove microbes and its antimicrobial property as it contains doxycycline, and affinity of doxycycline to bind to dental hard tissues. This parameter was not evaluated in this study and smear layer removal property of MTAD was not very significant as compared to 17 % EDTA solution and 10 % citric acid solution.The smear layer removal property of MTAD is attributed to 4.5% citric acid contained in this solution. The pH of citric acid is 2.5. The detergent used enhances the contact of MTAD to dentinal walls by reducing the surface tension. [49]

The application of 17% EGTA ( Ethylene glycol – bis beta amino ethyl ether) N, N, N, N – tetra acetic acid at concentration of 0.50 mol/litre is used for smear layer removal. Lot of research in medicine on chelating solutions to detoxify heavy metals that contaminate patients had been used.Yamamoto et al. reported that EGTA chelates only calcium ions where as EDTA chelates magnesium ions and calcium ions.Johnson et al. used EGTA to obtain extracellular environment totally free of calcium.Cruz-Filho et al. showed that action of 1% EGTA is similar to 15% EDTAC.[8]

In this study, 17% EGTA was effective in removing smear layer from cervical, middle and apical portion and was not effective in removing smear plugs. EGTA was not effective in opening of dentinal tubules.

The erosive effect of EGTA solution were found less when compared to 17 % EDTA solution and 10 % citric acid solutions.The smear layer property of EGTA solution was not appreciably significant when compared to EDTA and citric acid solutions for 5 minutes( fig. 15). EGTA specifically acts on calcium ions. EGTA has been used by various researchers at low concentrations (100µm to 1mM) when a medium free of calcium is required. Yamamoto et al. reported that EDTA chelates calcium and magnesium ions and EGTA chelates only calcium ions. [8]

In this study the dentin microhardness at middle third of root canal, halfway from the outer surface was recorded. EDTA treated samples gave mean value of 55.86 KHV and for EGTA treated tooth samples the mean values were 72.67 KHV. (Table –X) A significant difference was obtained between 17 % EGTA and 10 % citric acid (p value of 0.0001). (Table – XI). EGTA solution produced no effect on dentin microhardness

The reduction in dentin microhardness caused by EGTA was less. The hardness value for samples treated with EGTA solution were 72.00 MHV indicating the least demineralization produced when compared with EDTA, Citric acid and MTAD solutions. The dentin microhardness value was 72 .02 (Table –X) for the tooth samples treated by combined use of 5.25 % sodium hypochlorite and 17% EDTA for 5 minutes.

Alternating irrigation with 17% EDTA and 5.25 % sodium hypochlorite removed the inorganic as well as organic phase of dentin resulting in opening of dentinal tubular orifices, destruction of intertubular dentin and reduction in dentin microhardness. The combination regimen of sodium hypochlorite and EDTA produced less erosion and effectively removed smear layer from cervical, middle and apical portions ( fig. 19, fig.20 and fig. 21).

The unaffected root dentin has hardness value between 40 and 75 Kg $mm^{2}$ . Dentin hardness increases from root canal lumen towards the cement – dentinal junction, and values in the apical third are higher than in the middle and cervical sections of the root. The hardness of root canal wall is constant with Vickers hardness of 88.78 Kg $mm^{2}$ at the orifice of the root canal and 94.68 Kg mm-2 at the apex [6]

Quin et al. showed that if sodium hypochlorite was used again after EDTA or citric acid as final rinse, a marked erosion of root canal wall dentin occurs. In past decade, efforts were made on developing new irrigants and estsblishing new irrigation protocols.[71]

In the MTAD treated tooth samples the mean value was 53.58 KHV and in the10 % citric acid treated samples, values were 48.33 KHV. (Table – X).

A statistically significant difference in microhardness values were obtained when 17 % EDTA were compared with 10 % cirtic acid (p value – 0.0001). No siginificant differences in micro hardness was obtained between MTAD and 17 % EDTA (p value – 0.593). (Table –XI).

17% EDTA when used as final rinse was effective in removing smear layer from cervical and middle portions of root canal and caused erosion of intertubular dentin (fig. 12). Citric acid was effective in removing smear layer from apical portion of root canal (fig. 9 ) but caused reduction in dentinal microhardness ( fig. 22). MTAD was effective in cervical and middle portions but was least effective in apical portion ( fig. 18). EGTA caused least dentinal erosion but was not effective in removing smear plugs and opening of dentinal tubules (fig. 15).

Apical third of instrumented root canal found to have least degree of cleanliness obtained with the use of 17% EDTA, 17% EGTA and MTAD. The contact time and wetting of dentin walls by the irrigants seems to be the factor controlling the action of irrigants at the apical third of root canals.

The alternating use of 5.25% NaOCl and 17% EDTA facilitates adequate amount of cleanliness and smear layer removal ( fig .21). The protelytic action of 5.25% NaOCl on collagen matrix of dentin along with tissue dissolving capacity helps in superior cleaning action.The use of 10% citric acid caused disintegration of peritubular and intertubular dentin and resulted in substantial enlargement of the diameter of tubules. Citric acid also removed inorganic content from interfibrillar portion of intertubular dentin, thus causing reduction in inorganic content of dentin and reduction in microhardness.17% EGTA has specific action on calcium ions in hydroxyapatite crystals and caused least effect on mechanical properties of dentin.

When comparing the smear layer removal ability of root canal irrigants along with its impact on dentin structures, the irrigation regimen involving alternating use of 5.25% NaOCl and 17% EDTA resulted in adequate cleaning of instrumented root canals and also had least effect on dentin microhardness.

# SUMMARY AND CONCLUSION

**Summary -** The effect of 17% EDTA, 17 % EGTA. 10% Citric acid, MTAD and alternating use of 5.25 % sodium hypochlorite and 17 % EDTA as a final rinse on smear layer removal and dentin microhardness were determined. Sixty extracted mandibular pre molars were divided into five groups of 12 teeth each. All teeth were prepared by rotary instrumentation to an apical size of ISO 30 /0.06. During instrumentation, 5.25% sodium hypochlorite was used as initial rinse. Irrigants were delivered passively using 28 gauge side vented needles to within 1-2 mm short of working length. The specimens were split longitudinally and the changes in smear layer removal were evaluated using Scanning electron microscope. Dentin microhardness was evaluated by Vickers hardness tester. The obtained data were statistically analysed. 17 % EDTA was more effective in smear layer removal from cervical and middle third of instrumented root canals. The use of 10 ml volume of 17% EDTA for 5 minutes as final rinse resulted in opening of dentinal tubular orifices and destruction of intertubular dentin and reduction in dentin microhardness. EGTA solution caused least erosion of intertubular dentin and removed smear layer from cervical, middle and apical portions but was not effective in removing smear plugs. Use of 10 % citric acid as final rinse resulted in smear layer removal from cervical, middle and apical portions of root canal. Higher volumes of this solution over 1 minute application resulted in higher reduction in dentin microhardness than the alternating use of 5.25% sodium hypochlorite and 17 % EDTA. Biopure MTAD was effective in removing smear layer from cervical and middle portions of instrumented root canals but caused least effect in opening the tubules at apical portions. The irrigation protocol involving combined use of 5.25% sodium hypochlorite and 17% EDTA for less than 5 minutes effectively removed smear layer from cervical,middle and apical portion and created less erosion of intertubular dentin. 10% citric caused

the most reduction in microhardness followed by MTAD and 17% EDTA. The 17 % EGTA and alternating use of 5.25% sodium hypochlorite and 17% EDTA caused least reduction in microhardness when compared with the other irrigants.

**Conclusion –** Within the limitations of this invitro study it was found that the alternating use of 5.25% sodium hypochlorite and 17% EDTA appears to be the better irrigant when compared to 17% EDTA, 17% EGTA, 10% citric acid and MTAD solution in terms of smear layer removal ability and also has the least demineralization on dentin.

# TABLES

**Table I Smear layer removal scores after final rinse with 17% EDTA**

| N | Cervical | Middle | Apical | Hardness | Group I |
|---|---|---|---|---|---|
| 1 | 1 | 2 | 2 | 51.41 | EDTA |
| 2 | 1 | 1 | 2 | 55.18 | |
| 3 | 1 | 2 | 2 | 61.08 | |
| 4 | 1 | 1 | 2 | 58.01 | |
| 5 | 2 | 2 | 3 | 60.35 | |
| 6 | 1 | 2 | 3 | 60.32 | |
| 7 | 1 | 1 | 3 | 58.50 | |
| 8 | 1 | 2 | 2 | 52.30 | |
| 9 | 1 | 1 | 2 | 51.40 | |
| 10 | 2 | 2 | 3 | 54.10 | |
| 11 | 1 | 1 | 3 | 52.50 | |
| 12 | 2 | 2 | 2 | 55.18 | |

**Table II Smear layer removal scores after final rinse with 17% EGTA**

| N | Cervical | Middle | Apical | Hardness | Group II |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 3 | 68.67 | EGTA |
| 2 | 2 | 2 | 3 | 75.50 | |
| 3 | 1 | 1 | 3 | 85.50 | |
| 4 | 2 | 2 | 3 | 74.50 | |
| 5 | 1 | 3 | 2 | 77.40 | |
| 6 | 1 | 2 | 3 | 76.50 | |
| 7 | 2 | 2 | 3 | 67.60 | |
| 8 | 2 | 2 | 2 | 68.70 | |
| 9 | 2 | 3 | 3 | 69.50 | |
| 10 | 2 | 2 | 3 | 67.40 | |
| 11 | 2 | 3 | 3 | 66.30 | |
| 12 | 2 | 3 | 3 | 74.50 | |

**Table III Smear layer removal scores after final rinse with MTAD**

| N | Cervical | Middle | Apical | Hardness | Group III |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 3 | 50.08 | MTAD |
| 2 | 2 | 2 | 2 | 50.09 | |
| 3 | 1 | 2 | 3 | 53.20 | |
| 4 | 2 | 2 | 3 | 55.60 | |
| 5 | 1 | 1 | 3 | 57.60 | |
| 6 | 2 | 3 | 3 | 53.10 | |
| 7 | 2 | 2 | 3 | 52.60 | |
| 8 | 2 | 3 | 3 | 55.70 | |
| 9 | 2 | 3 | 3 | 51.20 | |
| 10 | 1 | 2 | 3 | 55.60 | |
| 11 | 2 | 2 | 2 | 57.60 | |
| 12 | 2 | 3 | 3 | 50.60 | |

**Table IV Smear layer removal scores after final rinse with 10% citric acid**

| N | Cervical | Middle | Apical | Hardness | Group IV |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 2 | 43.86 | Citric Acid |
| 2 | 1 | 1 | 2 | 45.50 | |
| 3 | 1 | 1 | 1 | 46.70 | |
| 4 | 1 | 2 | 1 | 51.30 | |
| 5 | 1 | 1 | 3 | 45.30 | |
| 6 | 1 | 2 | 1 | 44.50 | |
| 7 | 1 | 1 | 2 | 45.50 | |
| 8 | 1 | 2 | 2 | 46.70 | |
| 9 | 1 | 1 | 2 | 48.30 | |
| 10 | 1 | 1 | 1 | 50.40 | |
| 11 | 1 | 1 | 2 | 57.80 | |
| 12 | 1 | 1 | 1 | 54.20 | |

**Table V Smear layer removal scores after final rinse with 5.25% NaOCl & 17% EDTA**

| N | Cervical | Middle | Apical | Hardness | Group V |
|---|---|---|---|---|---|
| 1 | 2 | 2 | 2 | 70.30 | 5.25% NaOCl & 17% EDTA |
| 2 | 2 | 2 | 3 | 71.40 | |
| 3 | 2 | 3 | 3 | 73.40 | |
| 4 | 2 | 2 | 2 | 71.52 | |
| 5 | 1 | 2 | 3 | 71.40 | |
| 6 | 1 | 2 | 2 | 74.50 | |
| 7 | 1 | 2 | 3 | 70.40 | |
| 8 | 2 | 2 | 2 | 73.40 | |
| 9 | 1 | 2 | 2 | 72.40 | |
| 10 | 1 | 1 | 2 | 72.50 | |
| 11 | 1 | 3 | 3 | 70.60 | |
| 12 | 1 | 2 | 3 | 72.50 | |

## Smear Layer Removal –

The data obtained were discrete variables in scores Single comparison of taking groups together was performed with Kruskal – Wallis test of one way analysis for 5 groups and Chi square values were determined.

**Single Comparison taking groups together using Kruskal-Wallis Test**

**Mean and Standardard deviation of smear layer removal scores**

**Table –VI Mean, median and standard deviation of smear layer removal scores and one way analysis using Kruskal-Wallis test**

| Samples | EDTA | | EGTA | | MTAD | | Citric acid | | NaOCl & EDTA | | Chi-Square ($\chi^2$) | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±SD. | Median | Mean±SD. | Median | Mean±SD. | Median | Mean±SD. | Median | Mean±SD. | Median | | |
| Cervical | 1.25±0.45 | 1.00 | 1.75±0.45 | 2.00 | 1.75±0.45 | 2.00 | 1.00±.00 | 1.00 | 1.42±0.51 | 1.00 | 20.290 | .0001 |
| Middle | 1.58±0.51 | 2.00 | 2.33±0.65 | 2.00 | 2.33±0.63 | 2.00 | 1.25±0.45 | 1.00 | 2.08±0.51 | 2.00 | 23.283 | .0001 |
| Apical | 2.42±0.51 | 2.00 | 2.83±0.39 | 3.00 | 2.83±0.39 | 3.00 | 1.67±0.65 | 2.00 | 2.50±0.52 | 2.50 | 23.459 | .0001 |

Multiple comparisons of two groups taken at a time were performed using **Mann – Whitney U Test**. For all comparisons p value were fixed as less than 5 %.

**Table – VII Multiple comparisons of smear layer removal scores using Mann-Whitney U Test of Cervical third of root canal**

| Group Comparisions | | Mann-Whitney U Value | p value | Sig. |
|---|---|---|---|---|
| EDTA vs | EGTA | 36.000 | 0.016 | Sig |
| | MTAD | 36.000 | 0.016 | Sig |
| | Citric Acid | 54.000 | 0.070 | N.S |
| | NaOCl & EDTA | 60.000 | 0.397 | N.S |
| EGTA vs | MTAD | 72.000 | 1.000 | N.S |
| | Citric Acid | 18.000 | .0001 | H.S |
| | NaOCl & EDTA | 48.000 | 0.105 | N.S |
| MTAD vs | Citric Acid | 18.000 | 0.0001 | H.S |
| | NaOCl & EDTA | 48.000 | 0.105 | N.S |
| Citric Acid vs | NaOCl & EDTA | 42.000 | 0.014 | Sig |

**Table –VIII Multiple comparisons of smear layer removal scores using Mann-Whitney U Test of Middle third of root canal**

| Group Comparisions | | Mann-Whitney U Value | p value | Sig. |
|---|---|---|---|---|
| EDTA vs | EGTA | 30.500 | 0.008 | H.S |
| | MTAD | 30.500 | 0.008 | H.S |
| | Citric Acid | 48.000 | 0.105 | N.S |
| | NaOCl & EDTA | 41.000 | 0.031 | Sig |
| EGTA vs | MTAD | 72.000 | 1.000 | N.S |
| | Citric Acid | 16.500 | 0.001 | H.S |
| | NaOCl & EDTA | 55.500 | 0.265 | N.S |
| MTAD vs | Citric Acid | 16.500 | 0.001 | H.S |
| | NaOCl & EDTA | 55.500 | 0.265 | N.S |
| Citric Acid vs | NaOCl & EDTA | 21.000 | 0.001 | H.S |

**Table- IX Multiple comparisons of smear layer removal scores using Mann-Whitney U Test of Apical third of root canal**

| Group Comparisions | | Mann-Whitney U Value | p value | Sig. |
|---|---|---|---|---|
| EDTA vs | EGTA | 42.000 | 0.039 | Sig. |
| | MTAD | 42.000 | 0.039 | Sig. |
| | Citric Acid | 30.500 | 0.008 | H.S |
| | NaOCl & EDTA | 66.000 | 0.688 | N.S |
| EGTA vs | MTAD | 72.000 | 1.000 | N.S |
| | Citric Acid | 13.000 | 0.0001 | H.S |
| | NaOCl & EDTA | 48.000 | 0.090 | N.S |
| MTAD vs | Citric Acid | 13.000 | 0.0001 | H.S |
| | NaOCl & EDTA | 48.000 | 0.090 | N.S |
| Citric Acid vs | NaOCl & EDTA | 27.000 | 0.005 | Sig |

## Microhardness Test –

The data obtained were statistically analyzed using parametric one way analysis of variance ANOVA and significance was tested using f test. The p values was fixed at 5 %

**Parametric one way analysis of data using ANOVA Test**

**Table –X Parametric one way analysis of microhardness values using ANOVA test**

| Groups | Samples (N) | Mean ± SD. | Median | F Value | p value |
|---|---|---|---|---|---|
| EDTA | 12 | 55.860 ± 3.65 | 55.18 | 102.059 | 0.0001 |
| EGTA | 12 | 72.672 ± 5.65 | 72.00 | 102.059 | 0.0001 |
| MTAD | 12 | 53.580 ± 2.784 | 53.150 | 102.059 | 0.0001 |
| Citric Acid | 12 | 48.338 ± 4.289 | 46.700 | 102.059 | 0.0001 |
| NaOCl & EDTA | 12 | 72.026 ± 1.320 | 71.960 | 102.059 | 0.0001 |

Multiple comparisons of micro hardness between groups were evaluated using **Post Hoc Test Tuckey HSD analysis**

**Table –XI Multiple comparison of microhardness values using Post Hoc test by Tuckey HSD analysis**

| Group Comparisions | | Mean Difference | p value | Sig. |
|---|---|---|---|---|
| EDTA vs | EGTA | -16.8117 | 0.0001 | H.S |
| | MTAD | 2.2800 | 0.593 | N.S |
| | Citric Acid | 7.5225 | 0.0001 | H.S |
| | NaOCl & EDTA | -16.1658 | 0.0001 | H.S |
| EGTA vs | MTAD | 19.0917 | 0.0001 | H.S |
| | Citric Acid | 24.3342 | 0.0001 | H.S |
| | NaOCl & EDTA | 0.6458 | 0.994 | N.S |
| MTAD vs | Citric Acid | 5.2425 | 0.012 | Sig. |
| | NaOCl & EDTA | -18.4458 | 0.0001 | H.S |
| Citric Acid vs | NaOCl & EDTA | -23.6883 | 0.0001 | H.S |

**Table XII Mean and Standard deviation of Vickers hardness values**

| Groups | N | VHN Values (mean±SD.) |
|---|---|---|
| EDTA | 12 | 55.86 ± 3.65 |
| EGTA | 12 | 72.67 ± 5.65 |
| MTAD | 12 | 53.58 ± 2.78 |
| Citric Acid | 12 | 48.33 ± 4.28 |
| NaOCl & EDTA | 12 | 72.02 ± 1.32 |

# FIGURES


**Figure 1: Decoronated mandibular pre molars**


**Figure 2: : Irrigants Used in the Study – 10% citric acid, 5.25% NaOCl , 17% EDTA, 17% EGTA and MTAD**

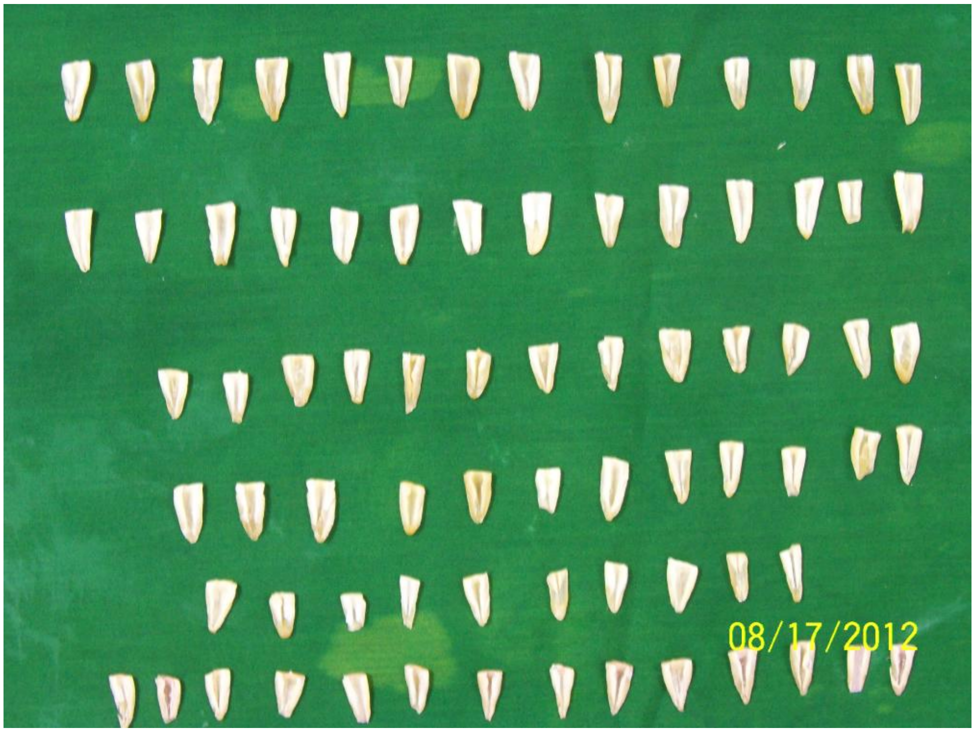


**Figure3: Longitudinal sections using chisel and mallet**


**Figure 4: JEOL JSM-5600LV Scanning electron microscope**

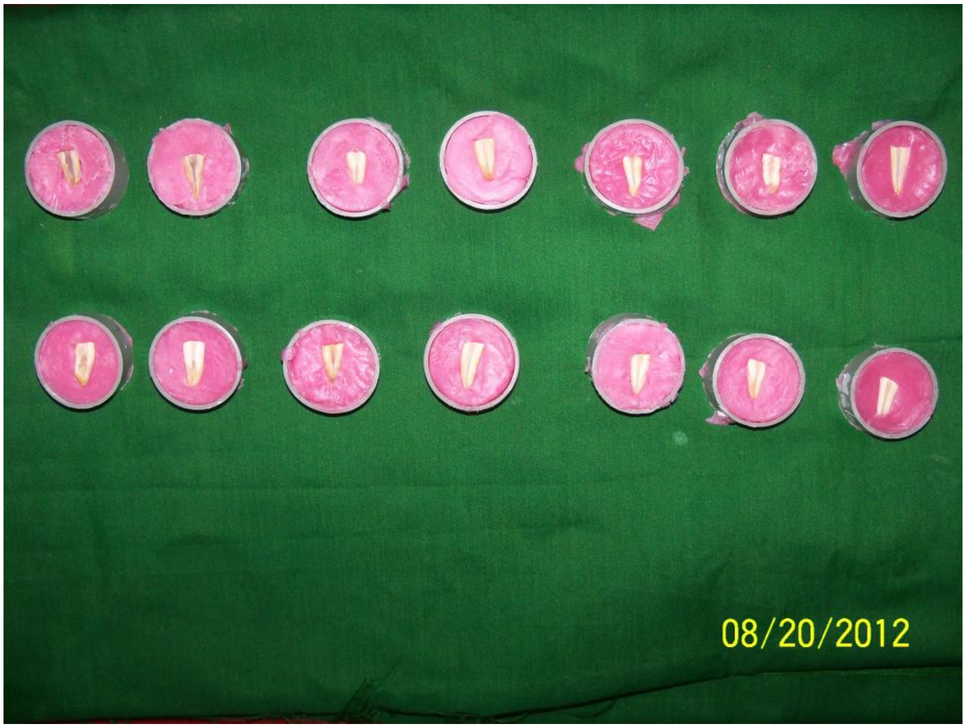


**Figure 5: Specimen embedded on cold cure acrylic resin**


**Figure 6: Vickers microhardness testing machine**

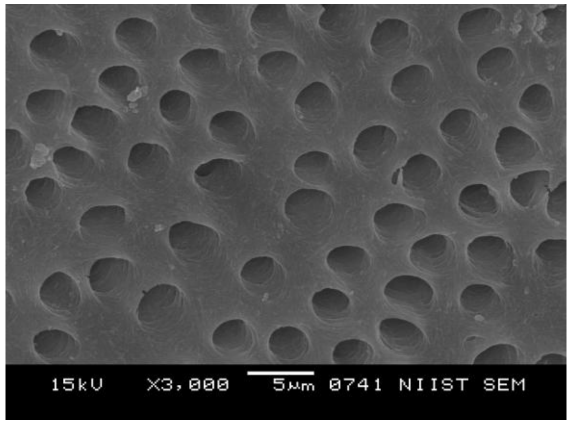


**Figure 7: Photo micrograph of cervical portion of root canal treated with Citric acid (X3000)**

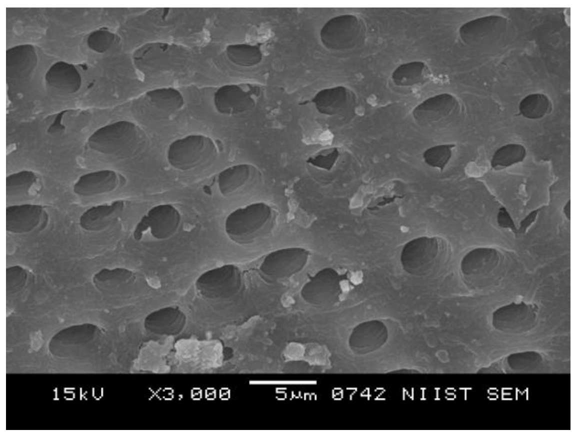


**Figure 8: Photo micrograph of middle portion of root canal treated with Citric acid (X3000)**

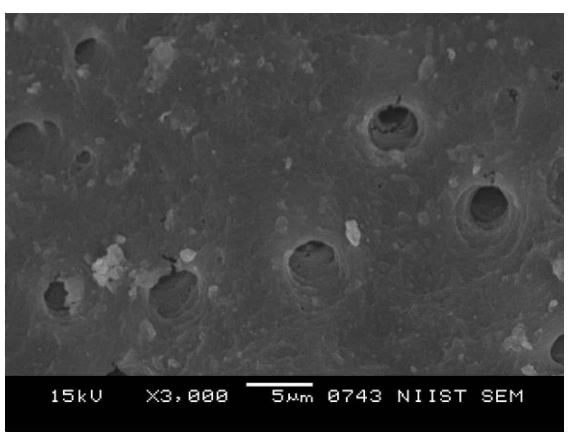


**Figure 9: Photo micrograph of apical portion of root canal treated with Citric acid (X3000)**

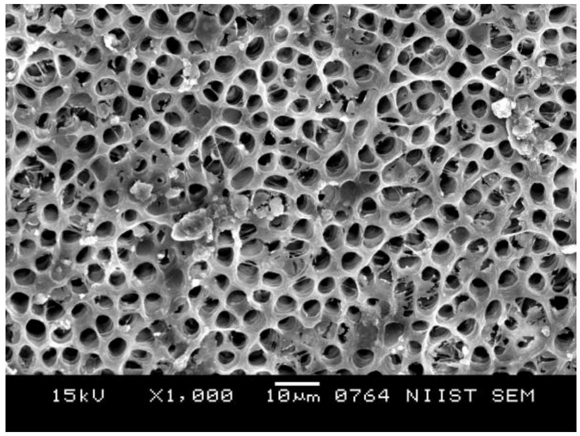


**Figure 10: Photo micrograph of cervical portion of root canal treated with EDTA (X1000)**

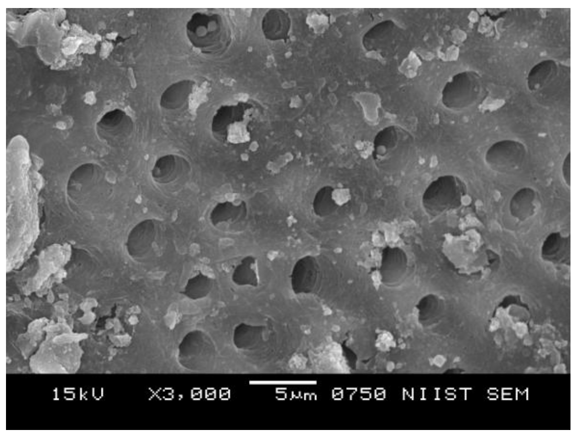


**Figure 11: Photo micrograph of middle portion of root canal treated with EDTA (X3000)**

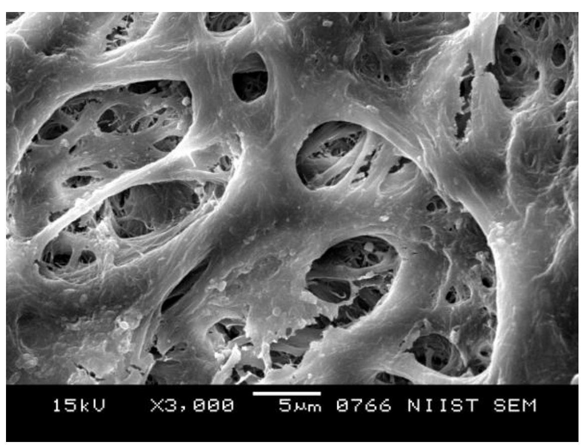


**Figure 12: Photo micrograph of apical portion of root canal treated with EDTA (X3000)**

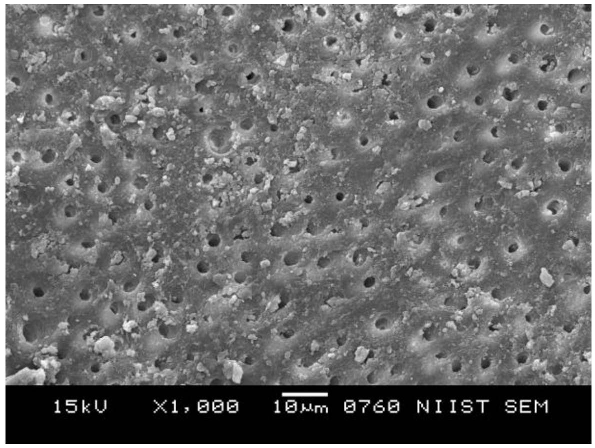


**Figure 13: Photo micrograph of cervical portion of root canal treated with EGTA (X1000)**

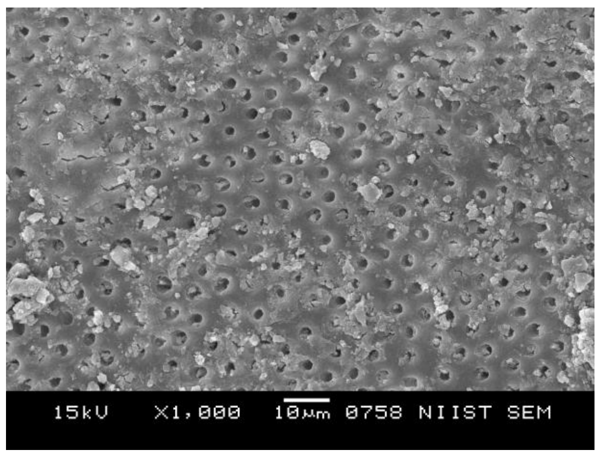


**Figure 14: Photo micrograph of middle portion of root canal treated with EGTA (X1000)**

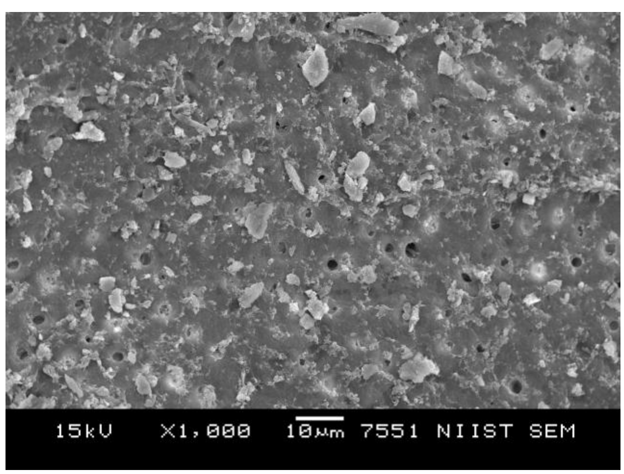


**Figure 15: Photo micrograph of apical portion of root canal treated with EGTA (X1000)**

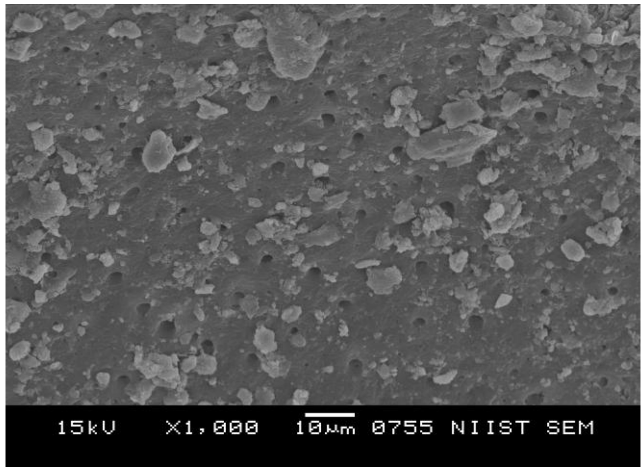


**Figure 16: Photo micrograph of cervical portion of root canal treated with MTAD (X1000)**

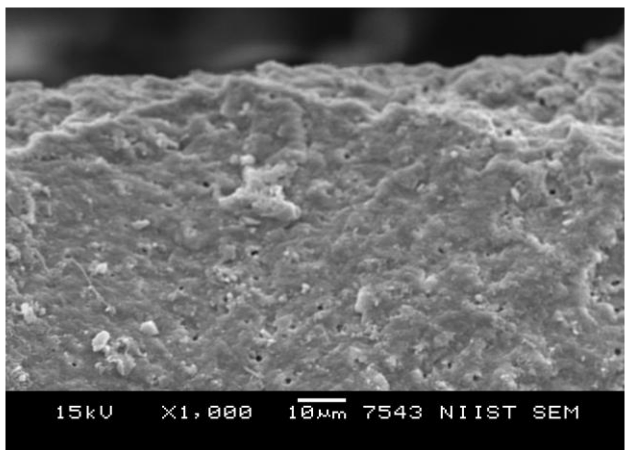


**Figure 17: Photo micrograph of middle portion of root canal treated with MTAD (X1000)**

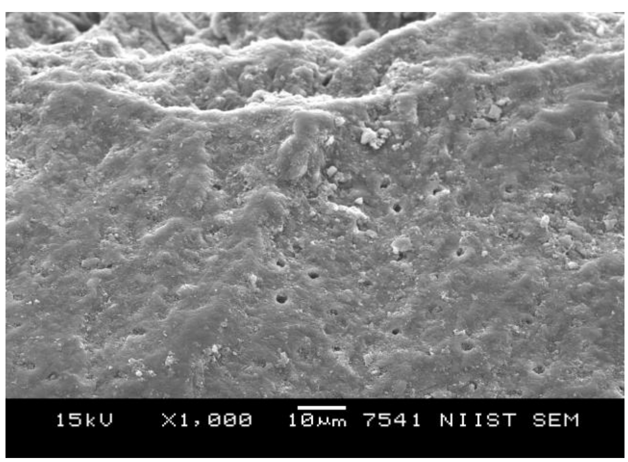


**Figure 18: Photo micrograph of apical portion of root canal treated with MTAD (X1000)**

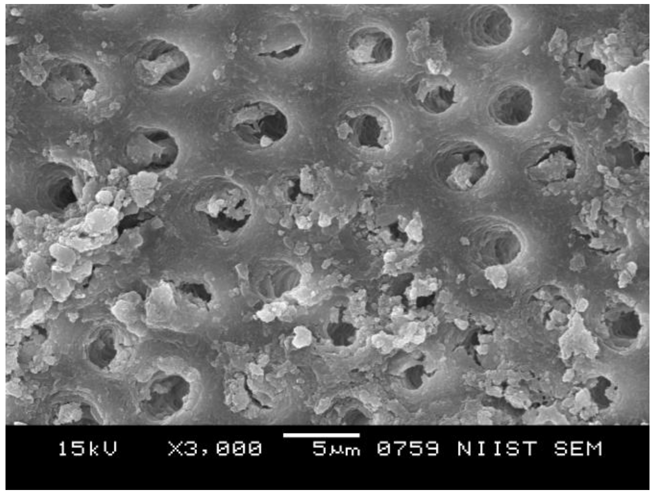


**Figure 19: Photo micrograph of cervical portion of root canal treated with NaOCl & EDTA (X3000)**

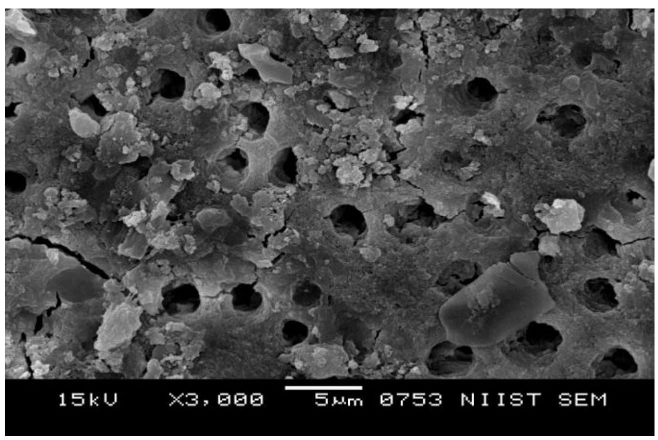


**Figure 20: Photo micrograph of middle portion of root canal treated with NaOCl & EDTA (X3000)**

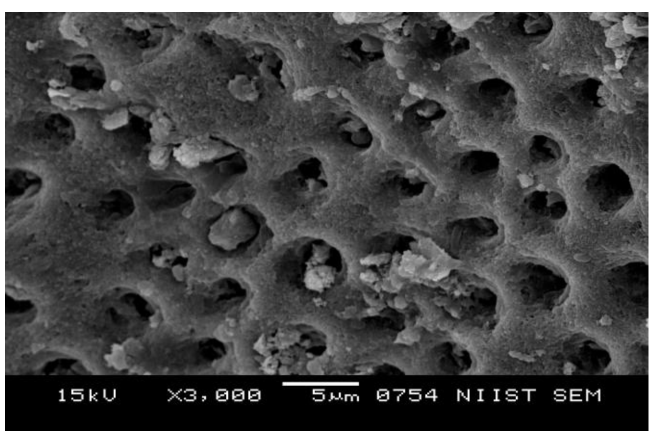


**Figure 21: Photo micrograph of apical portion of root canal treated with NaOCl & EDTA (X3000)**

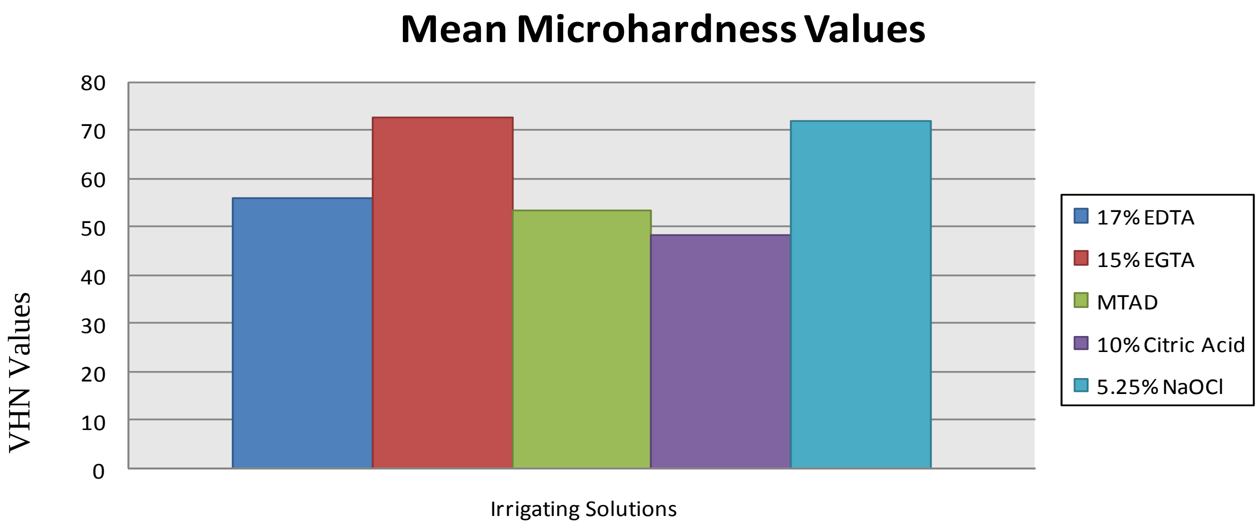


**Figure 22: Graphical representation of mean microhardness values**

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